PB-214 873

THE IDENTIFICATION AND MEASUREMENT OF CHLORINATED HYDROCARBON PESTICIDES IN SURFACE WATERS

A. W. Breidenbach, et al

Federal Water Pollution Control Administration
Washington, D. C.

1967

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U.S. DEPARTMENT OF THE INTERIOR
Federal Water Pollution Control Administration

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ERRATA

Page 36, Section VI, A, 1. line 5 - 1/1000 should read 1/100

Page 47, line 2 - valved should read valve

Page 64, A. ETHER SOLUBLES should read ETHER INSOLUBLES

Page 64, B. WATER SOLUBLES line 3 - ether solubles should read ether insolubles

Page 68, Reference 27, line 4 - Robret should read Robert

The IDENTIFICATION and MEASUREMENT of CHLORINATED HYDROCARBON PESTICIDES in SURFACE WATERS

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Mrs. Smith and Mr. Eichelberger are chemists working on the identification and quantification of organic compounds. Mr. Henke supervises the extraction and separation activities. Mr. Stierli, a registered professional engineer, serves as Chief of Engineering and Field Operations for the Activities.

ACKNOWLEDGEMENT

The authors wish to express their sincere appreciation to Dr. F. K. Kawahara, Consultant, Organic Chemistry, for his valuable assistance in the review of the several procedures and the editing of the manuscript. The excellent work of Mrs. D. Platt in preparing the final copy is gratefully acknowledged.

PREFACE

In September 1964 when this manual was first issued as an informal publication (PHS Publication #1241), it was recognized that anticipated progress in methods development would require periodic revision of the text. This issuance updates the previous effort and describes the methods currently employed in the Surveillance System laboratories of the Federal Water Pollution Control Administration.

Organic chemicals, as a group, have presented a special challenge to the laboratory because of the many thousands of such chemicals in use and the many complex mixtures of wastes produced in their manufacture. Specific identification and measurement of one class of organics, the chlorinated hydrocarbon pesticides, to a sensitivity of one microgram per liter or below is of particular concern.

The carbon adsorption method, developed over a decade ago, has been effectively employed in pesticide pollution studies. This method was pioneered and developed by a team of scientists at the Robert A. Taft Sanitary Engineering Center of the Public Health Service: F. M. Middleton, M. Ettinger, A. Rosen, G. Walton and H. Braus. While it is essentially a qualitative screening and continuous sampling technique when used on untreated surface waters, the method provides minimum quantitative values for measurement of specific substances. Most significant, the method has proved to be very useful for obtaining samples large enough for corroborative infrared and chromatographic identifications at low concentration levels.

Chromatography and chromatographic instrumentation have made possible the development and application of additional techniques by the Federal Water Pollution Control Administration's surveillance system laboratories. These newer techniques, applied to carbon adsorption extracts as well as discrete water samples, have been used to provide definitive identification and measurement of chlorinated hydrocarbon pesticides in surface waters. The rapid progress being made in methods research will surely result in continued modifications, improvements, and additions.

Further developmental work is going forward within the laboratory elements of the FWPCA and in other Federal agencies which have responsibilities associated with pesticides in the various segments of the environment. The Federal Committee on Pest Control provides guidance and coordination to interagency efforts in this problem area.

RICHARD S. GREEN, *Acting Chief*
Division of Pollution Surveillance
Federal Water Pollution
Control Administration

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I. INTRODUCTION

A. BACKGROUND

1. Federal Water Pollution Control Administration Surveillance System

The surveillance system [1] was established under the Public Health Service in October 1957 to implement that part of Public Law 660, as later amended, wherein the Secretary of the Department of Health, Education, and Welfare was authorized to collect and disseminate basic data on chemical, physical, and biological water quality insofar as such data relate to water pollution, prevention, and control. The system was expanded at the rate of 25 stations per year until it reached a total of 122 stations in September 1962. Six sampling stations were added during the following year. Two sampling stations were established in 1964 in the lower Mississippi main stem and one at Morgan City, Louisiana on the Atchafalaya. There are now a total of 131 sampling stations. Figure 1 shows the location of the stations.

Participants in the system include more than 176 state, local, and Federal water, sewage or other public utilities, health departments, industries, universities, state water pollution agencies, and resident engineers of Federal reservoirs. Active local participation is important in this operation. The state and local agencies perform most of the conventional chemical analyses and collect water samples for the more complex examinations. The FWPCA performs the more complex determinations at its Cincinnati laboratories and makes the results available to the various participants. The program as a whole is designed to assemble, examine, and interpret the facts which enable water pollution control agencies and others concerned to determine the scope and character of problems to be solved.

The analytical work of the surveillance system is devoted to characterization of surface water samples in six broad disciplinary areas. These are biological, microbiological and particulate matter, radiological, general chemical as well as physical properties and synthetic organic chemicals. Frequency of collection of the various discrete samples varies from several times per hour with automatic field instrumentation to once per month, depending on the type and purpose of the sample.

2. Organic Pollution

A very large variety of organic pollutants is known to be present in river water. These substances, present in small concentrations, may be carried to the stream

[1] Formerly the National Water Quality Network.

Figure 1. FWPCA Water Pollution Surveillance System Sampling Stations

in runoff, in domestic sewage or in industrial wastes. They are carried in solution and adsorbed to suspended solids. The 5-day biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrogen analyses and total carbon have been used successfully to aid in describing the degree and type of organic pollution as well as estimating oxidizability in the stream environment. These tests do not, however, serve as tools to identify and measure the specific organic compounds which are present in polluted water. The rapid and economical measurement of microgram and nanogram quantities of organic pollutants in water has been extremely difficult and impractical until very recently. Indeed, such minute quantities of specific substances, intermixed with a large variety of other interfering organic substances, have presented an extremely challenging and enigmatic analytical problem. Until recently, identification and measurement of most organic compounds in water in the parts per billion sensitivity range, has required an extremely large sample. Most methods, insofar as they have been developed, are not yet sufficiently sensitive to be of use with smaller samples.

The need for larger samples for exploratory work in characterizing organic pollutants was recognized over a decade ago and stimulated several years of research which ultimately produced the carbon adsorption method *(1) (2) (3) (4) (5) (6) (7)*.

B. CARBON ADSORPTION SAMPLES

This method, described in detail herein, uses the adsorptive capacity of activated carbon to concentrate organic materials from large water samples, measuring from 300 to 5000 gallons. This large sample permits corroborative identification by several analytical methods which can provide highly defensible identifications of specific substances. It must be noted, however, that the concentration values obtained for specific substances with this method must be considered as minimum values. First, the efficiency of the adsorption on and the desorption from carbon cannot be expected to be 100 percent for all compounds under widely varying physical and physico-chemical conditions in the water being sampled. Studies have shown recently, for example, that the adsorption of organics in streams on carbon is most efficient at flow rates and throughput volumes less than those which have been employed previously *(8) (9) (10) (11) (12)*. Secondly, until a means is available to gather organics adsorbed on the suspended solids as well as the organics in solution from the large sample, the determined concentration values (e.g., microgram per liter of water) must be considered low. The increased yields per unit volume of water from low flow equipment are primarily due to the longer contact time. However, they may result in part from trapping of and subsequent desorption from some of the suspended solids. Pesticides have been identified in carbon adsorption samples in the past *(13) (14) (15) (16) (17)*.

C. BOTTLED SAMPLES

1. Water Samples

Water grab samples of one liter to one gallon are useful. The grab sample, properly taken, contains water in which organics are dissolved as well as suspended solids on which organics are adsorbed. The absolute weight of the organic material is

so small in most grab samples as to restrict the approach used for identification to noninfrared analysis. However, if the organic substance present can be detected, it can usually be measured at very low levels. Thus, in the microgram per liter concentration range, carbon adsorption samples provide enough material so that the potential for corroborated identification exists. Grab samples are most useful for rapid and highly sensitive measurement and, in addition, supply further data on which the identification can be based. A number of workers have identified pesticides in water grab samples (*18*) (*19*) (*20*) (*21*).

2. Bottom Samples

Pesticides have been identified in silt (*22*) and soils (*23*) (*24*) (*25*) (*26*). The water in a stream is closely related to the solids suspended in it, as well as the solids deposited on the bottom. Solids accumulated on the bed of a stream may contain larger quantities of organics than the water above. Extensive studies on the Lower Mississippi River have shown this to be true in the case of certain chlorinated pesticides. Therefore, it is equally important to analyze the bottom samples as well when assessing the degree of a pollution problem.

II. SAMPLE COLLECTION

Carbon adsorption samples and discrete one-liter samples are taken for the identification and measurement of organic substances in surface water.[2] These sampling approaches have been combined with sensitive thin layer chromatographic and gas chromatographic methods for pesticide analysis. The details of the sampling techniques employed in the laboratories of the FWPCA surveillance system are outlined below.

A. THE CARBON ADSORPTION METHOD (CAM)

This technique, developed in 1951 (*1*), was applied to raw surface waters in a pilot study in 1956 and has been in routine use in the surveillance system since 1957. Since that time the sample collection aspects of the technique have undergone considerable refinement. Pertinent details of the sampling equipment are included in Appendix One.

1. Preparation of the Carbon Adsorption Cartridge (CAC)

The carbon adsorption cartridge consists of a Pyrex glass pipe three inches in diameter and 18 inches in length packed with two types of carbon. To pack the vertically oriented cylinder are added successively 4.5 inches of 4 x 10 mesh carbon,[3] nine inches of 30 mesh carbon,[4] and an additional 4.5 inches of 4 x 10 mesh carbon. The cylinder is packed full but not tightly. The coarse carbon at each end of the cartridge aids in preventing clogging by mud and silt from turbid waters. However, cartridges employing all fine (C–190) carbon at low flow rates and reduced throughput volumes are being used successfully (see Appendix One). The cartridge is shipped to the field station and installed in the appropriate sampling system.

Sample volumes of 300 and 5000 gallons (*1*) (*27*) (*28*) of water taken at rates of 0.03 and 0.5 gpm have been used successfully; however, sampling efficiency can be increased by the use of smaller volumes and lower flow rates. Efficiency is further increased with the low flow rate system using a column containing only 30 mesh carbon. The reproducibility (*29*) and effect of the variables of total throughput and rate of flow through the carbon (*10*) have been the subject of intense study

[2] Although these methods are employed currently for untreated surface water the methods are also applicable to ground water and treated water.

[3] Cliff Char 4 x 10 mesh (Cliffs Dow Chemical Co., Marquette, Mich.)

[4] Nuchar C–190 (West Virginia Pulp and Paper Co., New York, N.Y.)

by Booth. A sampling system for lower flow rates and lower throughput volumes was designed by Castelli and Booth (*30*). Field tests show that sampling efficiency is greatly improved under these conditions (*31*) (see Appendix One).

After the desired quantity of water is sampled, the carbon cartridge is disconnected and returned to the laboratory for analysis (see Figure 19, Appendix One).

2. Precautions Necessary to Prevent Accidental Contamination of Carbon

The affinity of carbon for organic substances requires that supplies of carbon be protected from extraneous sources of contamination. For example, carbon can adsorb organic substances such as paint vehicles and insecticides used for pest control from the air. Therefore, the carbon is stored and processed in an area adequately protected from such sources of contamination. As an additional precaution, the ventilating, heating, and air conditioning systems for the laboratories in which carbon adsorption samples are processed are completely isolated from all other laboratories. All carbon is obtained from the manufacturer in sealed metal drums. Obviously, spraying with pest control chemicals is not permitted in these areas. Carbon blank determinations supplement these precautions (see Section V).

B. DISCRETE BOTTLED SAMPLES

1. Water Samples

Approximately one liter (940 ml) of water is collected in each of two wide-mouthed glass bottles equipped with screw caps fitted with Teflon liners. The bottle should be filled to about ½ inch from the top. The mouth of the bottle must be wiped clean before securing the cap to prevent leaking. These two bottles represent one sample. Plastic bottles (polyethylene) are not used because traces of plasticizer are leached from the plastic by the water and can be a source of analytical interference. Moreover, organics from the water are adsorbed on the plastic. It has been suggested that high grade Teflon (Nalgene) bottles may be satisfactory for this use; however, the cost is prohibitive at present. Many investigators avoid the use of glass sample bottles because breakage in shipment frequently causes loss of sample. This is overcome by the use of relatively inexpensive, expanded, polystyrene foam shipping containers molded to fit the bottle (see Figure 2).

2. Bottom Samples

Samples may be collected from the stream bottom with a St. Anthony Falls type device (*32*). The sample is placed in a 1-quart bottle such as described in B.1. above. The bottle should be about ½ filled. The samples may be coarse or fine gravel, sand, silt, or clay.

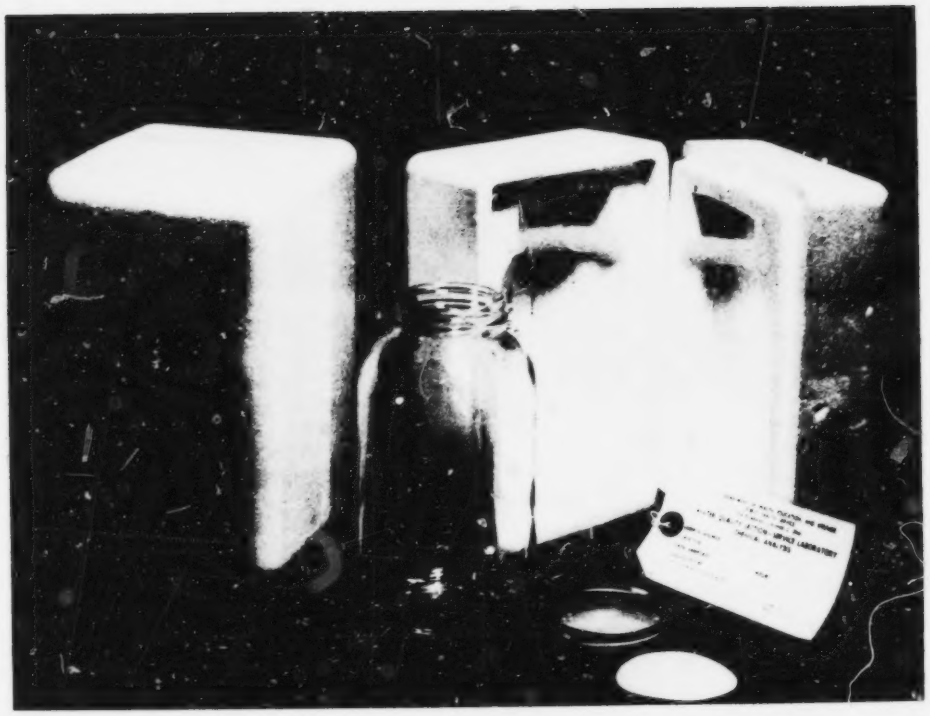


Figure 2. Screw-cap (Teflon-lined), Glass Sample Containers and Expanded Polystyrene Cartons

3. Preparation of the Container

Bottles are rinsed successively with chromate cleaning solution, running tap water, distilled water, and finally several times with redistilled solvent (e.g., acetone, hexane, petroleum ether, chloroform). Caps and liners are washed with detergent, rinsed with tap water, distilled water and solvent.

III. PREPARATION OF SAMPLES PRELIMINARY TO GAS CHROMATOGRAPHIC ANALYSIS

After a carbon adsorption or grab sample is received, it is logged and all pertinent data (source, date sampled, date received, quantity of water sampled) are recorded (see Figure 3).

A. CARBON ADSORPTION SAMPLES

1. Treatment of Carbon

a. Drying the Carbon

The carbon is dried by spreading it on stainless steel trays in an oven at 40°C. for about two days[5, 6] (see Figure 4). If there is a backlog of dried carbon samples on hand they are sealed in solvent-rinsed, one-gallon, wide-mouthed tin cans and held for further treatment.

b. Extraction of the Carbon

Large scale Soxhlet extractors are used to extract the dried carbon. The quantity of carbon used in the sampling cartridge is accommodated by the extractors (see Figure 5).

(1) *Packing the extractors*

To prevent carbon fines from passing into the boiling flask, the bottom of the extractor is packed with about three inches of pre-extracted glass wool.[7] The wool is wetted with chloroform. The dried carbon sample is added and packed by tamping so that it just fits the extractor. If carbon is packed too tightly, siphoning will be severely hindered. The frequency of siphoning is controlled at 2 cylinder volumes per hour. Siphoning is not always automatic and application of compressed air to the vent of the extractor is sometimes necessary.

(2) *Chloroform extraction*

The Soxhlet is filled with redistilled chloroform and siphoned over twice. More chloroform is added, if necessary, and the sample is extracted continuously for

[5] Copper or brass trays may also be used. Galvanized metals or aluminum react with wet carbon. Metal coated with high quality Teflon has also been suggested.

[6] The air circulated through the oven is prefiltered through carbon to prevent contamination from the atmosphere.

[7] Oily organic substances are first removed from glass wool by extraction with chloroform.

35 hours.[8] After the extraction is completed, the bulk of the chloroform is siphoned and blown over into the boiling flask. The flask is removed from the system, the extract concentrated to about 250 ml by distillation,[9] and filtered through solvent-washed filter paper into a 300-ml Erlenmeyer flask. The solvent is evaporated to approximately 20 ml on a steam bath with a jet of clean, dry air.[10] The contents of the flask are transferred to a tared glass vial and the remaining solvent evaporated at room temperature in a hood *without* a jet of air. The carbon chloroform extract (CCE) is judged dry when the chloroform odor can no longer be detected.[11] The weight of the residue is obtained.

[8] Longer extraction times may be used but 35 hours (24 for ethanol) is considered optimum. Booth (*29*) has confirmed this point.

[9] Two-zone Glas-Col heating mantles are used to prevent overheating and scorching of the sample.

[10] First the compressed air is cleaned and dried by directing it through a bed containing carbon and a drying agent such as calcium chloride.

[11] A trace of chloroform is retained by the CCE. This is, however, insignificant in most samples and no correction is necessary. In very large fluid samples correction may be necessary and can be accomplished using a procedure developed by Mashni (*33*). Unfortunately, it is not practical to do this on a routine basis for large numbers of samples.

STANDARD ORGANIC ANALYSIS OF CARBON ADSORPTION SAMPLES

STATION NO. _____ SOURCE _____

SAMPLE NO. _____ LOCATION _____

DATE _____ TO _____ TYPE OF WATER _____

RECEIVED _____ COMPOSITE _____ QUARTER _____

FLOW IN GALLONS _____ ; LITERS _____

EXTRACTION DATA

EXTRACT	GRAMS	P.P.B.	PER CENT	DATE EXTRACTED
CHLOROFORM				
ALCOHOL				
TOTAL				

SEPARATION OF CHLOROFORM EXTRACT

SOLUBILITY SEPARATION	P.P.B.		PER CENT	
ETHER INSOLUBLES				
WATER SOLUBLES				
NEUTRALS				
		NEUTRALS		NEUTRALS
ALIPHATICS				
AROMATICS				
OXYS				
LOSS				
TOTAL				
WEAK ACIDS				
STRONG ACIDS				
BASES				
LOSS				
TOTAL				

REMARKS:

SEC 169a
(6-54)

FRONT

WEIGHT OF SAMPLES IN GRAMS

CHLOROFORM EXTRACT	ALCOHOL EXTRACT

SOLUBILITY SEPARATION		CHROMATOGRAPHIC SEPARATION
CHLOROFORM EXTRACT		NEUTRAL FRACTION
ETHER INSOLUBLES	WATER SOLUBLES	ALIPHATICS
WEAK ACIDS	STRONG ACIDS	AROMATICS
BASES	NEUTRALS	OXYS

BACK

Figure 3. Laboratory Data Card

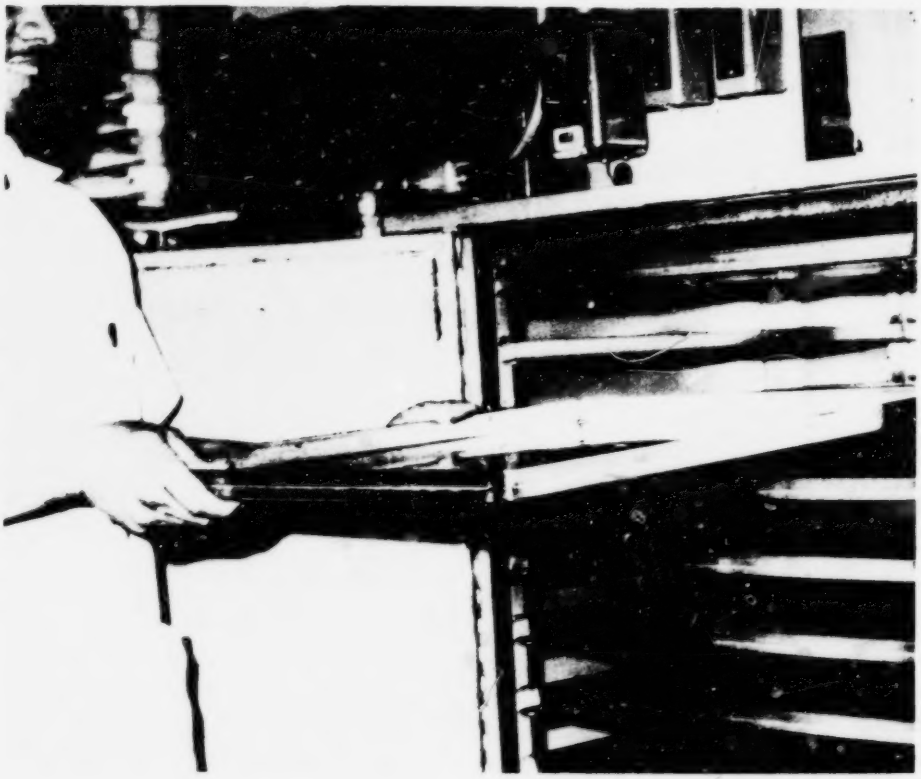


Figure 4. Carbon Drying Oven

(3) *Ethanol extraction*

This step is not used in routine pesticide analysis. The residual chloroform is removed from the carbon by blowing pre-cleaned warm air through the carbon (in place in the Soxhlet) and exhausting chloroform vapors through the hood. In order to do this the Soxhlet is removed from the hood and the carbon shaken loose to facilitate movement of air through it. The Soxhlet, still containing the carbon, is returned to the hood with the glass plate cover removed. A hose from a heated air manifold (approximately 60° C.) is attached to the bottom of the siphon tube and the air is blown up through the carbon for three to four hours or until it is dry (see Figure 6). Alternate methods,[12, 13] may be employed but this procedure has proved much less hazardous, consumes less time and requires less supervision. Ethanol (95%) is added to the dried carbon and the extraction is carried out in the manner described for the chloroform step. Extraction is terminated after 24 hours.[8]

Concentration of the carbon alcohol extract (CAE) is begun as described for the chloroform extract. However, the drying, started on a steam bath with a jet of air,

---

[12] The carbon is removed from the Soxhlet and dried in the oven as in III, A, 1, a. This procedure requires about 48 hours. Adequate ventilation is required to remove hazardous chloroform vapors.

[13] The residual chloroform may be leached from the carbon by pouring alcohol over it. Proceed as follows: Siphon twice and distill until 68° C. is reached. Repeat a second and third time, distill to 77° C. and begin extraction. Add alcohol if necessary. The distillate (68° to 77° C.) may be used for the initial leaching of the carbon in succeeding extractions. This procedure requires about 4 hours.

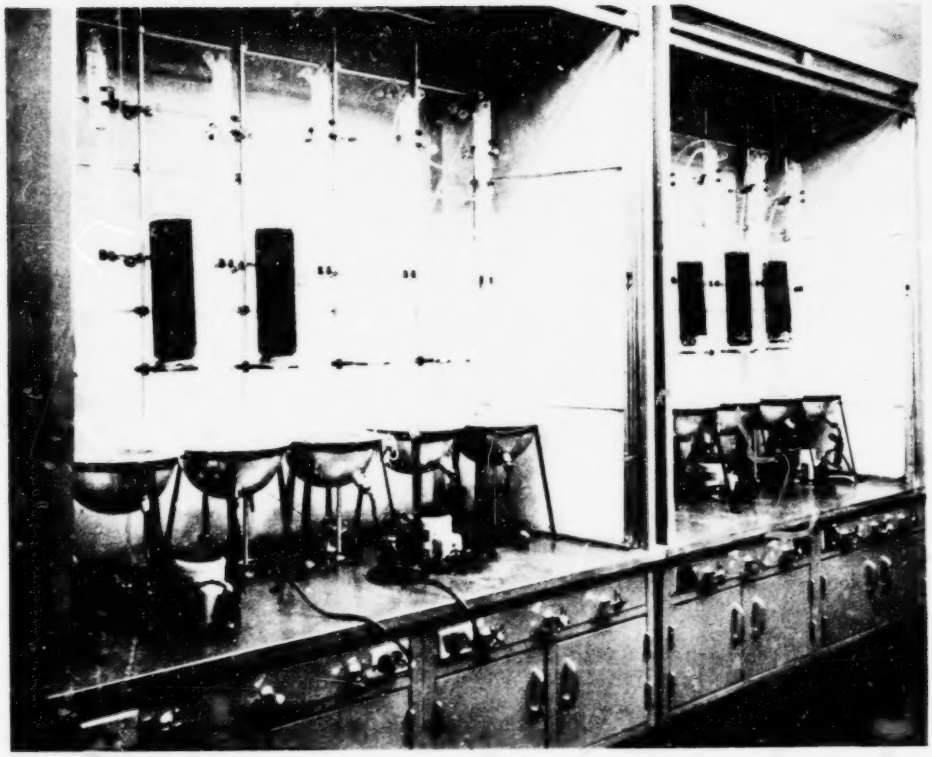


Figure 5. Soxhlet Extractors

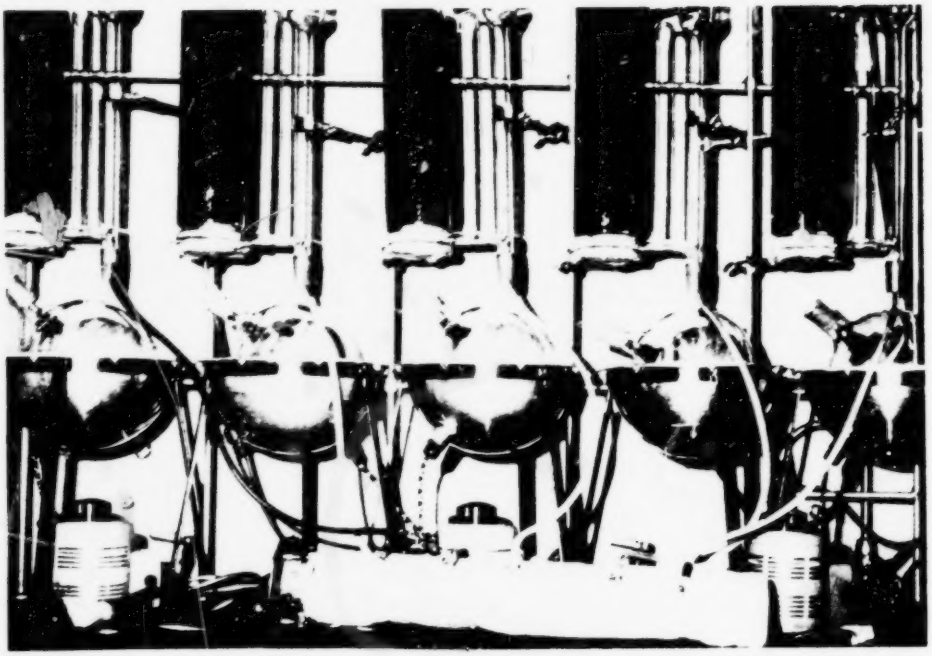


Figure 6. Removal of Residual Chloroform from Carbon

is continued in an oven at 75° C. until weight change of successive weighings at 72-hour intervals is less than 1%.

2 Preliminary Separation of CCE (27) (34) (35) (36) (37)

The procedure described under paragraph 2a is employed when all classes of compounds are of interest. When only chlorinated hydrocarbon pesticide information is sought, the procedure 2b is used.

The separation techniques and analytical procedures described below are carried out as quantitatively as possible. Careful attention is directed at details of quantitative transfer, controlled evaporation and accurate weighing. Ether extractions are carried out in a hood.

a. Procedure for General Organic Analysis[14]

(1) *Solubility separation* (See Figure 7)

(a) Weigh out approximately 0.5 gram of CCE (a) in a 50 ml beaker. As little as 0.1 g or less can be used; however, the percentage error increases as the weight of the aliquot decreases.

(b) Add about 1 ml of methanol to the sample and stir. Dissolve sample in 30 ml of ether and stir. If there is appreciable insoluble material, filter through a sintered-glass funnel under vacuum. The residue (b) is the ETHER INSOLUBLE fraction (EI). Transfer the residue back to the 50-ml beaker using methanol, evaporate on a steam bath, cool and weigh.

(c) Transfer the ether solution (c) to a 125-ml separatory funnel. (Do not use stopcock lubricants—use only glass or Teflon stopcocks). Extract three times with 15 ml portions of distilled water and combine extracts in a tared 125-ml Erlenmeyer flask. Evaporate water (d) to dryness on a steam bath with a jet of air, cool and weigh. This is the WATER SOLUBLE fraction (WS).

(d) Extract the ether solution (e) remaining in the funnel three times with 15-ml portions of dilute HCl (5%). Set the ether layer (f) aside and make the HCl extract (g) strongly basic (pH > 10) with NaOH pellets or 25% NaOH solution. After cooling, extract three times with 15-ml portions of ether, combine in a 125-ml

[14] It is recognized that use of strong acids and bases as described in the solubility separation may cause condensation, hydrolysis or decomposition reactions to occur. Thus, specific determinations may require alternate techniques.

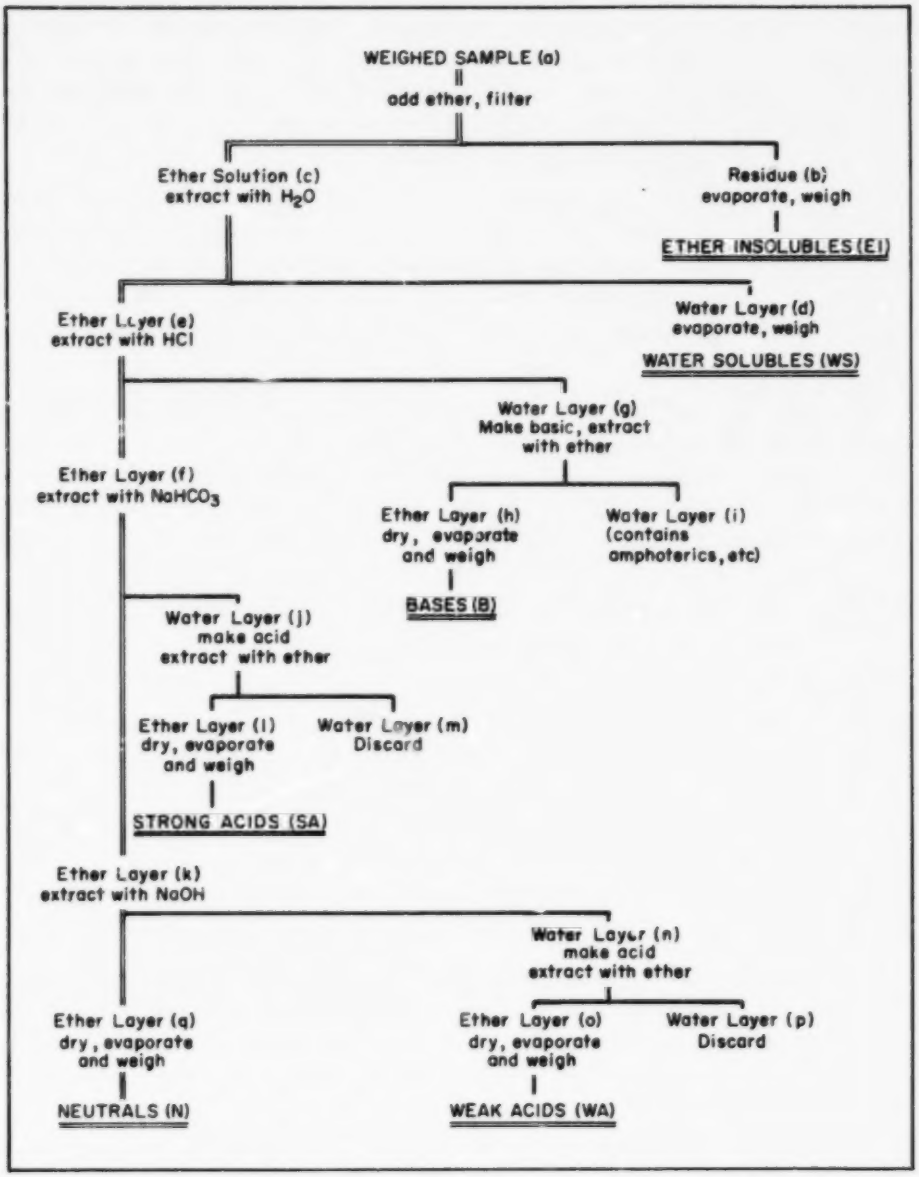


Figure 7. Flow Scheme for Solubility Separation of CCE

Erlenmeyer flask, dry, evaporate and weigh. The residue (h) is the BASIC FRACTION (B). Discard the water layer (i).[15]

[15] The basic water layer (i) remaining after ether extraction may contain some amphoteric and some water-soluble substances. If these substances are of interest, a special plan for analysis should be set up (34).

(e) Extract the ether layer (f) three times with 15-ml portions of $NaHCO_3$ (5%). Set the ether layer (k) aside and make the $NaHCO_3$ extract (j) strongly acidic (pH < 2) by careful addition of concentrated HCl. After cooling, shake vigorously to release CO_2. Extract three times with 15-ml portions of ether, combine in a 125-ml Erlenmeyer flask, dry, evaporate, and weigh. The residue (l) is the STRONG ACID fraction (SA). Discard the water layer (m).

(f) Extract the ether solution (k) three times with 15-ml portions of NaOH (5%) and once with distilled water. *Caution:* Emulsions may form during this step. Set the ether layer (q) aside and make the NaOH extract (n) strongly acidic with concentrated HCl. After cooling, extract three times with 15-ml portions of ether, combine in a 125-ml Erlenmeyer flask, dry, evaporate and weigh. The residue (o) is the WEAK ACID fraction (WA). Discard the water layer (p).

(g) The ether solution (q) contains the NEUTRAL fraction (N). Place in a 125-ml Erlenmeyer flask, dry, evaporate and weigh.

Ether solutions are dried by pouring over a two-inch column of anhydrous sodium sulfate followed by ether rinses. Alternately one may add sodium sulfate (10 g) to the flask and filter off the sulfate after standing overnight.

(2) *Chromatographic separation of the neutral fraction* (See Figure 8)

(a) Pack activated silica gel [16] in a Pyrex glass column 20 mm in diameter to a height of 10 cm.

(b) Weigh[17] the neutral sample in a 10-ml beaker and dissolve in a minimum amount of ether. Add sufficient silica gel to adsorb the sample. Evaporate the ether gently.

(c) Wet the column with about 20 ml of iso-octane and add the sample when the last of the 20 ml reaches the surface of the adsorbent. Rinse the beaker with iso-octane and add the rinsings to the column. The beaker should be rinsed several times with iso-octane and each succeeding eluent when the eluent is added to the column.

(d) Elute the ALIPHATIC fraction (AL) with 85 ml of iso-octane[18] (a) and collect in a tared 150 ml beaker. The eluent should be added carefully with a medicine dropper so as to disturb the surface of the adsorbent as little as possible. A slow, dropwise, elution rate is desirable. It may be necessary to apply mild pressure to obtain a satisfactory rate.

(e) After the level of the iso-octane has reached the surface of the adsorbent, replace the receiving beaker with another tared 150 ml beaker. Elute the AROMATIC fraction (AR) with 85 ml of benzene (b) and collect.

(f) After the liquid level of the benzene has reached the surface of the adsorbent, replace the receiving beaker with another tared 150 ml beaker. Elute the OXYGENATED fraction (OXY) with 85 ml of a 1:1 mixture of methanol and chloroform (c), and collect.

---

[16] Davison Code 950–08–08–226 (60 to 200-mesh). Davison Chemical Co., Baltimore 3, Maryland.
[17] It is convenient to retain for future reference about 5 mg of the neutral fraction.
[18] Limited investigation suggests that hexane may also be used.

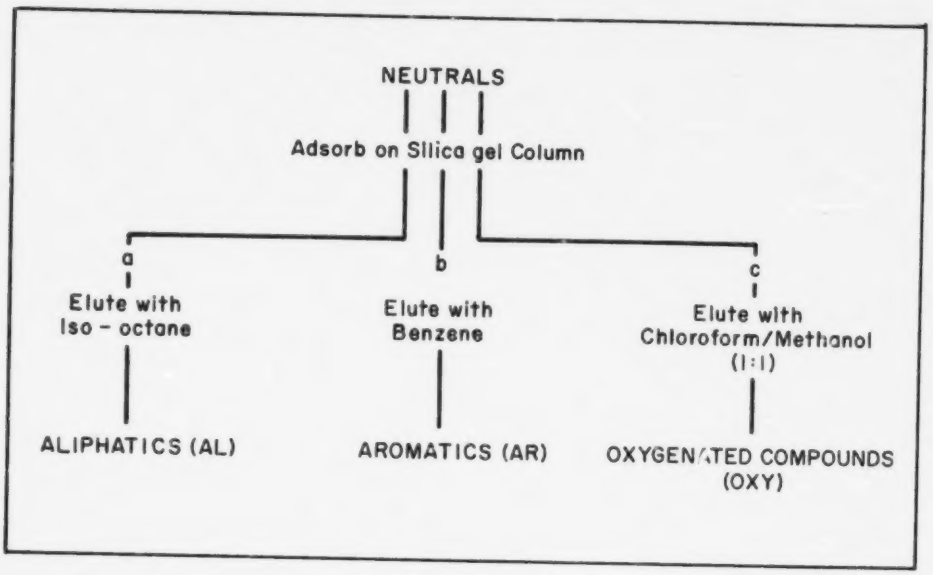


Figure 8. Flow Scheme for Chromatographic Separation of Neutrals

(g) Carefully evaporate the three fractions on a steam bath with a jet of dry clean air, cool, and weigh. The beakers should be removed from heat and air before the solvent is completely evaporated.

(3) *Infrared spectra*

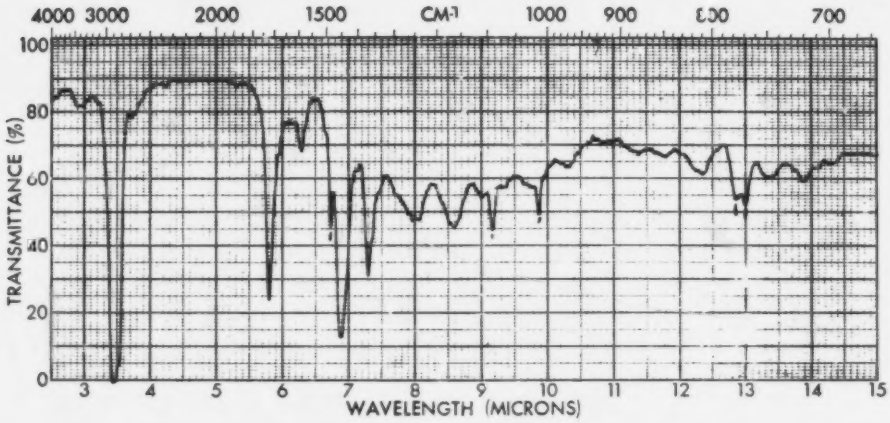
(a) Chloroform extracts—infrared spectra are obtained on selected fractions that have unusual physical characteristics or odors. In analyses directed at chlorinated hydrocarbons, the IR spectra of the aromatic fraction is run. See Appendix Three and Figures 9 and 10.

(b) Ethanol extracts—infrared spectra, only, are obtained on the alcohol extracts. The percent recovery of the CCE and concentration ($\mu g/l$) are calculated and recorded. The percent and concentration of the various fractions, obtained through the separation of the CCE are calculated and recorded.

b. Column Chromatographic Separation of CCE (Alternate Procedure)

This procedure may be employed to separate the aromatic fraction more rapidly from the CCE when this fraction is of interest. Since most chlorinated hydrocarbon pesticides, including lindane, DDT, DDD, DDE, dieldrin, endrin, aldrin, heptachlor and heptachlor epoxide, are found in the aromatic fraction, this step is employed when pesticide data are needed and other substances are of lesser interest. However, laboratory tests indicate that three other pesticides, methoxychlor and methyl and ethyl parathion (organophosphorus compounds) occur in the oxy fraction as might be expected from their chemical structure.

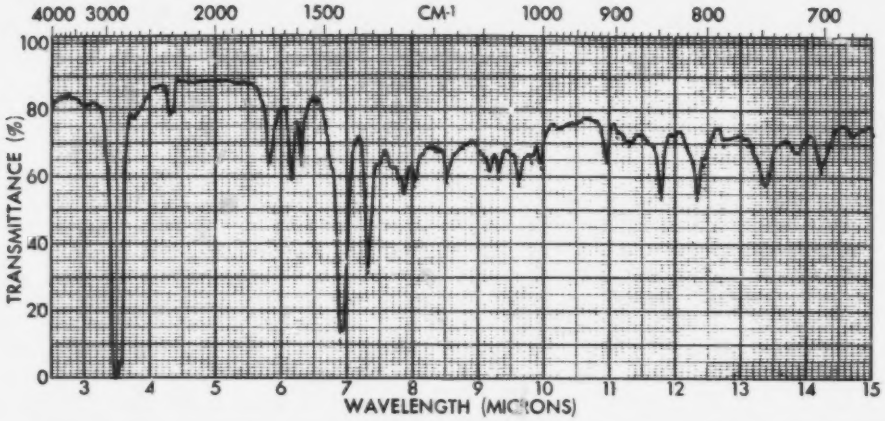
The use of this alternate procedure does not preclude the isolation of the



SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
SAMPLE YAKIMA RIVER, WASHINGTON	PURITY	1.	C.C.E.
	PHASE	2.	AROMATIC FRACTION
	THICKNESS	DATE	
		OPERATOR	

SPECTRUM NO. SAMPLE

Figure 9. Infrared Spectrum of Aromatic Fraction of CCE Sample Supporting Chromatographic Identification of DDT



SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
SAMPLE SAVANNAH RIVER, SOUTH CAROLINA	PURITY	1.	C.C.E.
	PHASE	2.	AROMATIC FRACTION
	THICKNESS	DATE	
		OPERATOR	

SPECTRUM NO. SAMPLE

Figure 10. Infrared Spectrum of Aromatic Fraction of CCE Sample Supporting Chromatographic Identification of Dieldrin

other classes of organics, because the longer, generally applicable solubility separation can be employed with the third (chloroform-methanol) eluate if necessary.

This chromatographic separation technique for the CCE is identical to that used in separation of the neutral fraction. In this case, however, 0.5 gram of the CCE (or less) is weighed in a 50-ml beaker, dissolved in a minimum quantity of chloroform and added to just enough silica gel to adsorb the dissolved sample. The chloroform is gently evaporated and the sample adsorbed on the silica gel is added to the column as described in Section A.2.a.(2) above. The aliphatic fraction, the aromatic fraction, and a complex residue, eluted with chloroform-methanol, are obtained.

c. Thin Layer Chromatographic (TLC) Separation of Pesticides[19] From Aromatic Fraction of CCE (*38*).

(1) *Preparation of plates*

Layers of silica gel 0.25 mm thick are prepared on 200 x 200 mm glass plates. A thin slurry is prepared of 30 g of silica gel G in about 60 ml of water and spread over five plates with the aid of a variable thickness spreading device. The plates are allowed to stand five minutes, then dried in an oven for 60 minutes at 110° C. and stored in a desiccator for future use.[20]

(2) *Preparing the solvent system*

The developing solvent, carbon tetrachloride, is added to the chamber to a depth of 10 mm (approximately 200 ml). Two filter paper wicks, one on each side of the chamber, are placed so that one end contacts the solvent. After the lid is in place, the chamber is allowed to equilibrate for one hour.[21]

(3) *Spotting of plates*

Marks are made near the edge of each plate at distances of 1.5 and 11.5 cm above the bottom edge to define the spotting line and the point at which the solvent front has moved to 10 cm.

The entire aromatic fraction of the CCE is dissolved in benzene in a 15-ml centrifuge tube and made up to 0.5 ml. A 50-ul aliquot or one-tenth of the aromatic fraction in benzene is spotted[22] (see Figure 11).

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[19] Samples of very high total organic content (CCE or grab) may require additional clean-up, e.g., the Mills florisil column procedure (*39*) (*40*).

[20] Layers of varying thickness have been investigated. Layers thicker than 0.25 mm produced no better separation and thinner layers were consistently less uniform. Aluminum oxide plates 0.25- and 0.50-mm thick were also prepared. The range of R_f values for the pesticides spotted was considerably smaller than those observed with silica gel plates using the same solvent system. Thus separation was not as good. However, alumina plates do have special applications. For example: pesticides that show a tendency to streak on silica gel (chlordane, toxaphene) produce single spots on alumina. Also, it is possible to separate dieldrin and endrin, which on silica gel normally have approximately equal R_f values.

[21] Many different developing systems, both multi-component and single component, have been investigated. In general, the single component systems showed much more consistent results than did the multi-component systems. The single component system which showed the best separation of all pesticides investigated was carbon tetrachloride. The range of R_f values of pesticides was the best of all systems investigated.

[22] The aromatic fraction of the CCE is generally 5 to 15 mg, or approximately 1 to 5% of the CCE. The average pesticide content of most river waters sampled by the carbon adsorption method has been estimated to be about 2% of the aromatic fraction. Fifty microliters or 10% of the aromatic fraction yields enough material for good pesticide peaks in gas chromatographic analysis, when separated by thin layer chromatography.

It is necessary to direct a very gentle stream of clean, dry air on the point of spotting to keep the diameter of the spot less than 1.0 cm. The use of air should be kept to a minimum. A typical plate for pesticide analysis may contain up to nine sample spots. The samples may be spotted in duplicate; one for elution and gas chromatographic analysis and one for possible corroborative visual identification on the sprayed portion of the plate. In addition, pesticide standards are spotted on the plate.

(4) *Development*

The spotted plate is placed in the chamber so that the bottom edge is in contact with the solvent and the lid is replaced. When the solvent reaches the upper reference line (10 cm), the plate is removed and the solvent allowed to evaporate. The spots are made visible by spraying the developed plate with a dye or a chromogenic agent. Two spraying procedures are described below.

(a) RHODAMINE B METHOD

The areas of the plate containing the unknown samples are masked with a glass plate and the center area containing the pesticide standards is sprayed with a fairly heavy coat of Rhodamine B. The plate is allowed to dry completely (about 5 min.) and is examined under UV light. The pesticides are seen in natural light as purple spots on the pink background, but are seen much more readily under UV light where they appear as quenched areas on the fluorescent background.

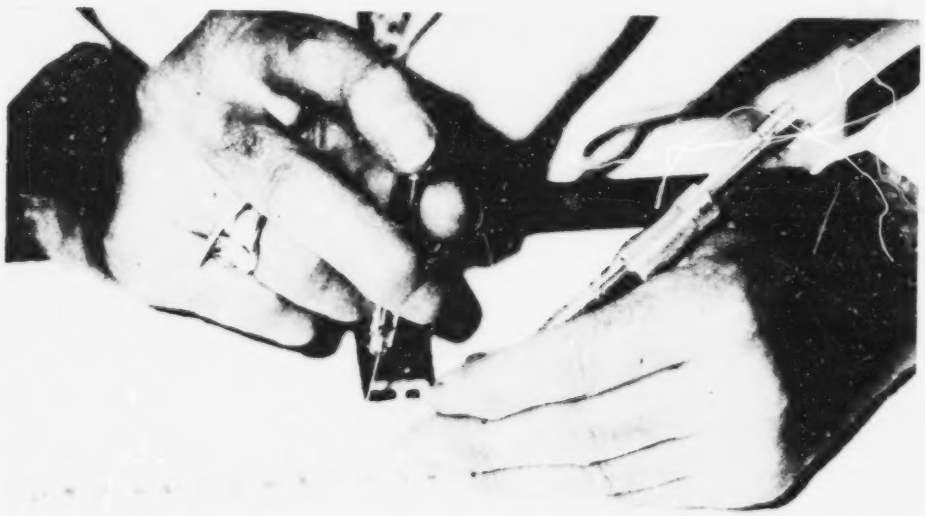

Figure 11. Spotting of TLC Plate

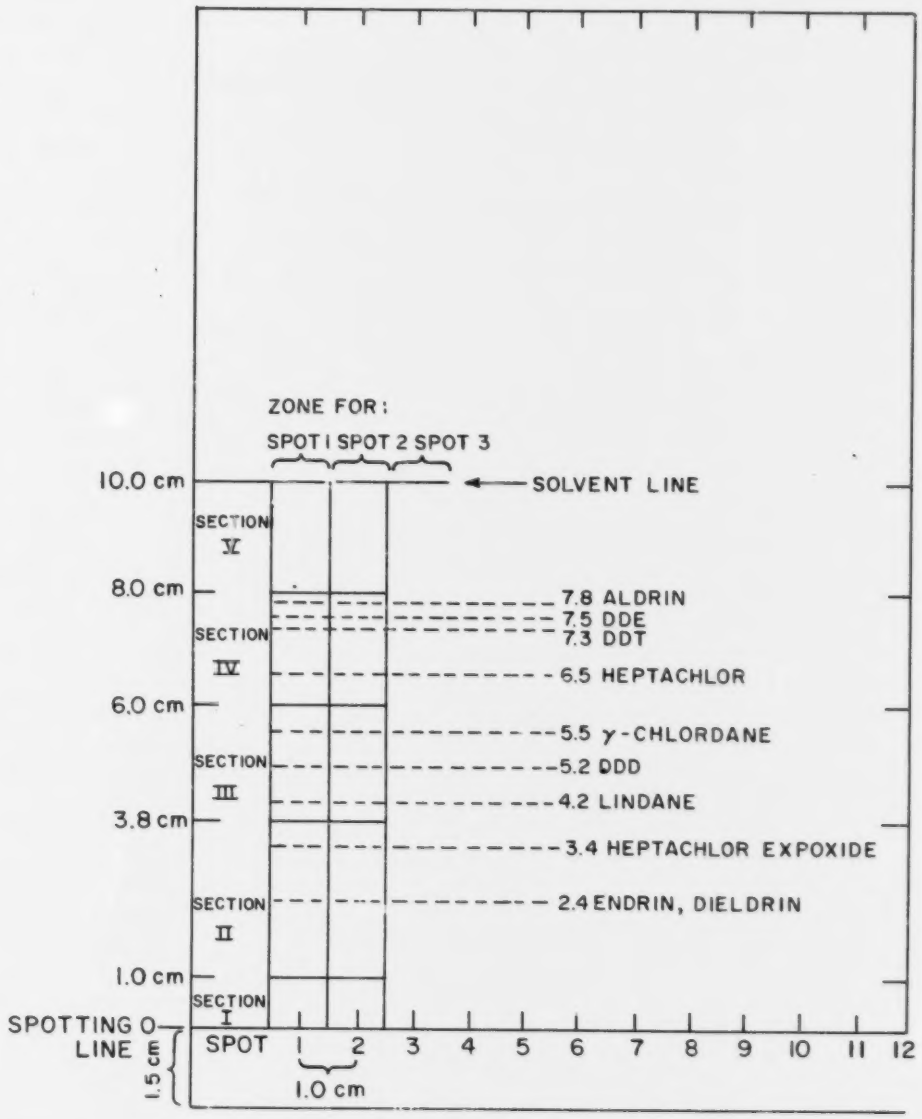

Figure 12. Diagram of Designation of Sections in the Cleanup and Separation of CCE-Aromatics on Silica Gel Layers

The vertical zones of travel for pesticides present in the unknown samples will be respectively the same as those of the sprayed standards which are visible. From this information, the vertical zone of travel for each sample spot is divided into five horizontal sections. The sections are identified with Roman numerals as shown in

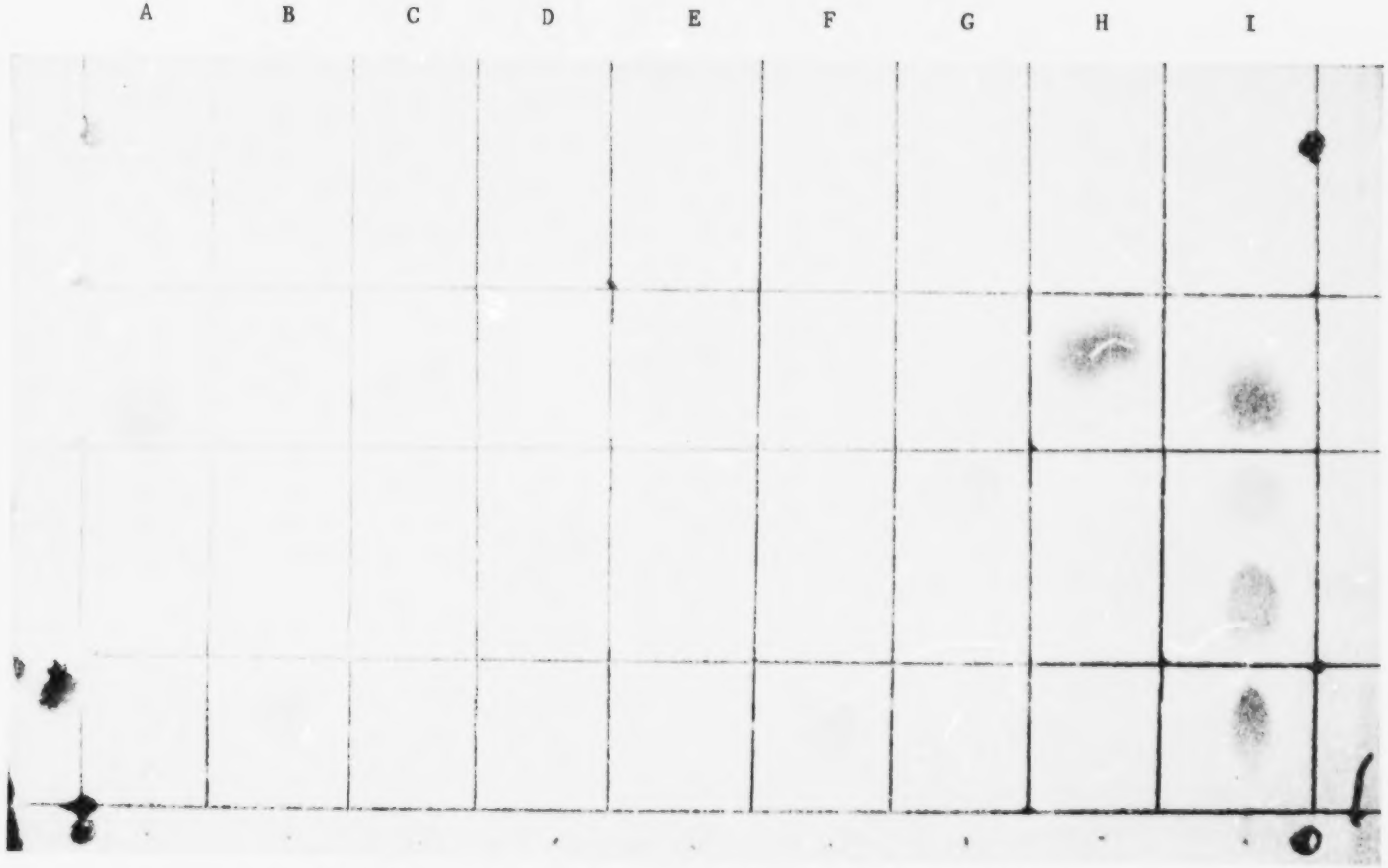

Figure 13. Photograph of a Developed Thin Layer Plate. A. DDT, B. Endrin, C. Heptachlor, D. Benzene Hexachloride, E. Aldrin, F. Dieldrin, G. DDD, H. DDE, I. Mixed Standard.

Figure 12.[23] A photograph of a developed plate is shown in Figure 13. These horizontal sections are determined by the R_f values of the standard pesticide spots. The silica gel in each section is scraped loose from the plate with a spatula. With the aid of vacuum, the silica gel, first from the periphery of the spot and then from the area of the spot itself, is drawn into an eye dropper which is plugged at the tip with glass wool (Figure 14). The material adsorbed on the silica gel in each eye dropper is eluted quantitatively into a graduated 15 ml centrifuge tube with 5 to 10 ml of acetone and subjected to gas chromatographic analyses.

The following pesticides have been investigated and can be seen at the 10 μg level under UV light after spraying with Rhodamine B:

endrin	toxaphene
dieldrin	DDE
DDT	chlordane
aldrin	parathion
lindane	methyl parathion
heptachlor	ovex
heptachlor epoxide	tedion
methoxychlor	DDD

The R_f values for most of these compounds are listed in Table 1.

The application of Rhodamine B as a spray reagent has definite advantages over silver nitrate spray. With Rhodamine B, the exact position of the pesticide on the plate can be determined without destroying the pesticide. Therefore, using a selective solvent (ether/petroleum ether 1:1), the pesticide can be eluted from the silica gel while the Rhodamine B is retained. However, these advantages apply only when the entire plate, samples as well as standards, is sprayed.

[23] Repetitive testing of standard pesticides and the subsequent resolution of nine chlorinated hydrocarbon pesticides resulted in the designation of the illustrated sections.
The eluate from Section V was repeatedly analyzed by gas chromatography to determine if chlorinated pesticides occurred in it. None of the pesticides studied occurs in this Section. Hence, it is not analyzed on a routine basis.

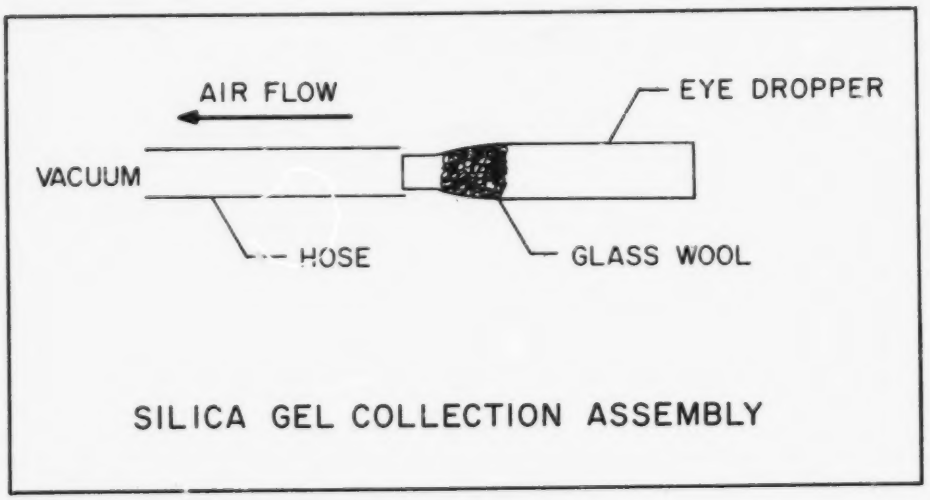

Figure 14. Silica Gel Collection Assembly

(b) SILVER NITRATE METHOD

An alternate spraying technique (*41*) employing silver nitrate may be used for locating the pesticides on thin layer plates. The samples may be spotted in the manner described above. However, silver nitrate destroys the pesticide and, thus, if gas chromatographic analysis is to follow and corroborative identification on the plate is desired, the samples must be spotted in duplicate. To accomplish this, two series of samples are spotted along with standards. An internal dye,[24] previously standardized against pesticides, is also spotted to determine the zones of travel. The zones are marked and the sections for the samples to be analyzed by gas chromatography are scraped and eluted as above.

The samples and standards remaining on the plate are then sprayed with silver nitrate, dried, and exposed to UV light until spots appear. Chlorinated pesticides appear as brown to black spots.

Chlorinated and non-chlorinated pesticides may be detected by exposing the untreated, dry plate to bromine vapor for 30 seconds, drying for 30 seconds, and spraying with a fluorescein solution and finally with silver nitrate (*42*). Exposure to UV light for 4 to 7 minutes causes chlorinated pesticides to appear as brown to black spots and other pesticides as yellow to white spots on the tan background.

B. DISCRETE BOTTLED SAMPLES

1. Extraction of Pesticides

a. Extraction From Water

(1) Semi-Automatic Extraction (*43*) (*44*)

Equipment for semi-automatic liquid-liquid extraction of chlorinated hydrocarbon pesticides as well as other organic compounds from water was developed to be compatible with the sample bottle currently employed in this laboratory (see Figures 15 and 16). It consists essentially of two parts. The first part is a cylindrical impeller housing and bar magnet having an inlet port and four outlet ports. The second part is a plug which fits the mouth of the sample bottle and provides the means of reclamation of the solvent when the extraction is completed.

Extraction is accomplished by placing the impeller in the sample bottle containing approximately 850 ml water, and adding 50 ml of a hexane-benzene mixture (9:1). The sample bottle is capped and inverted on a magnetic stirrer so that the impeller may operate on a flat surface. To reduce magnetic attraction between the bottle cap and the bar magnet in the impeller, the center is cut out of the bottle cap. An aluminum insert with a Teflon liner is used to seal the opening.

When the stirrer is operating, the rotating motion of the impeller creates a vertical vortex in the sample and draws the hexane-benzene mixture into the central inlet port. Small bubbles of the solvent are ejected from the four radially located outlet ports. The bubbles rise through the outer portion of the sample to the surface where they collect and recirculate as long as the magnetic stirrer is operating. When the extraction has been carried out for 30 minutes, the bottle is removed from the stirrer. The cap is replaced by the plug used in reclamation of the solvent. The

[24] The R_f of the pesticides will vary, since temperature and humidity conditions are not controlled. However, with the aid of the dye mixture (see Appendix Three) which has been previously standardized against pesticide spots, the sections may be adjusted to compensate for these deviations.

plug is held in place by a pressure device and air under pressure is introduced through an inlet. This air forces the hexane-benzene mixture out of a tube which extends into the solvent phase just above the solvent-water interface. As much as possible of the solvent is collected and measured in a 50-ml graduated cylinder.[25]

The water remaining in the bottle is poured into a 1000-ml graduated cylinder and its volume measured and recorded.

(2) Separatory Funnel Extraction

[25] Recoveries of the pesticides using the semi-automatic extractor range from 77 to 95%.

Figure 15. Semi-automatic Liquid-Liquid Extractor

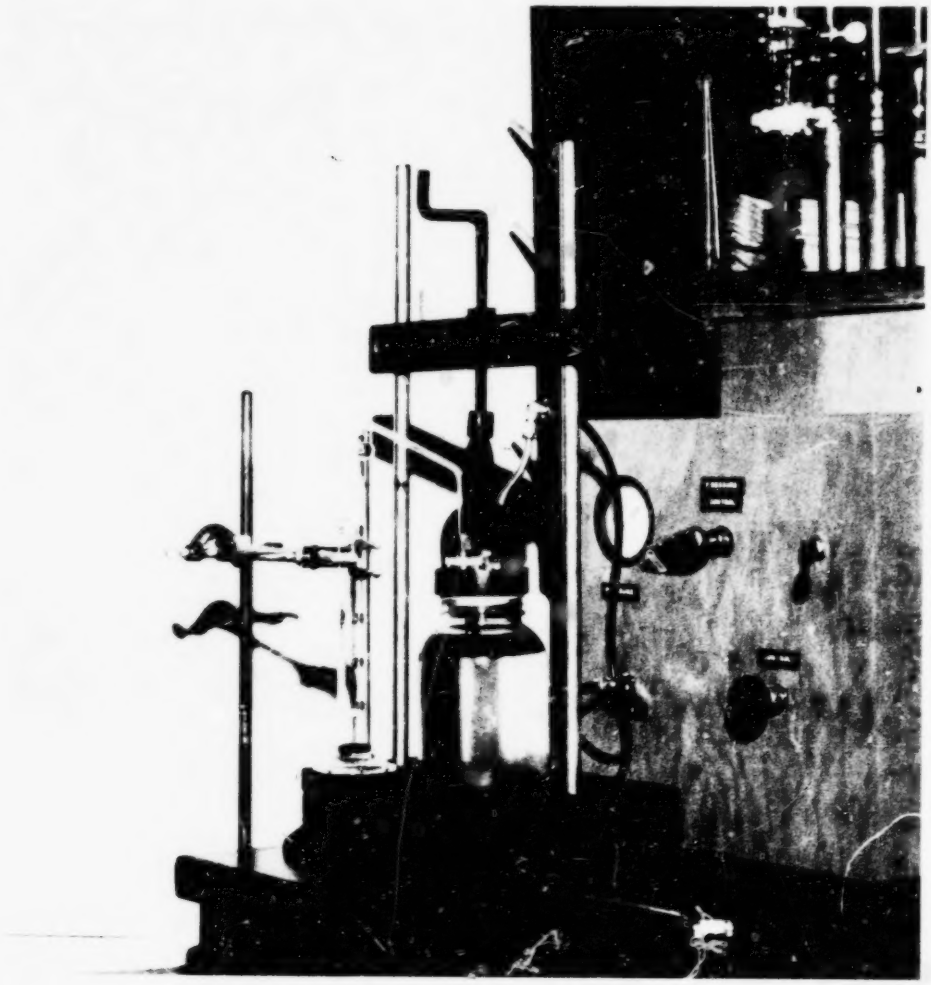

Figure 16. Solvent Recovery Apparatus

The entire measured water sample (approximately 1 liter) is drained into a 2-liter separatory funnel equipped with a Teflon stopcock and is extracted successively with 100, 50, 50, 50, and 50 ml of redistilled hexane.[28]

The drained sample container is rinsed with three 50-ml aliquots of hexane. The first two rinse volumes serve as the first extraction volume (100 ml) for the sample. The third rinse serves as the second (50 ml) extraction volume.

The sample is shaken moderately for four minutes. (Vigorous shaking may cause severe emulsions, particularly in waters of high organic content and/or high turbidity.) The extracts are combined in a 300-ml Erlenmeyer flask and dried by

[28] Other solvents such as carbon tetrachloride, chloroform, and ethyl ether-petroleum ether (1:1) may be used (51) (20) (47).

pouring through a two-inch column of anhydrous sodium sulfate. The column is rinsed three times with approximately 5 ml of hexane and the rinsings are added to the extract.[27]

b. Extraction From Bottom Samples

(1) Drying the Sample

The excess water is decanted and the sample is spread in a pyrex dish (8" wide x 12" long x 2" deep). The sample is air dried at room temperature for about 4 to 5 days. Many pesticides are volatile and may be lost if drying is carried out at elevated temperatures or for an excessive length of time.

The dried sample is ground with a porcelain mortar and pestle to a uniform particle size.

(2) Extraction

The sample is divided by mixing and quartering until a sub-sample of about 100 grams is obtained. The sample is weighed in a 100-ml beaker.

The extraction is carried out in a soxhlet extractor (see Figure 17). Glass wool (about 1 inch deep) is packed in the bottom of the extraction chamber (40 x 150 mm). The weighed sample is added and an additional wad of glass wool is placed on the top. The sample is then extracted using 200 ml of hexane-acetone (9:1) for about 8 hours. The extraction may be carried out overnight or longer as may be necessary for heavily contaminated samples.

Several alternate extraction methods are described in the literature (*25*) (*49*) (*50*).

2. Concentration of Extract

a. Water Sample

The extract (approximately 50 ml) obtained by semi-automatic extraction is transferred to a 100-ml beaker and evaporated on a 40°C warm water bath to approximately 4 ml using a very gentle stream of clean, dry air. It is then transferred with rinsing to a 15-ml graduated centrifuge tube and evaporated to an appropriate volume for spotting on a TLC plate.

The extract (approximately 300 ml) obtained by separatory funnel extraction is transferred to a Kuderna-Danish evaporator and concentrated to approximately 4 ml. It is then transferred to a 15-ml graduated centrifuge tube and evaporated to an appropriate volume for spotting on a TLC plate.

b. Bottom Sample

The extract (approximately 200 ml) is evaporated in the extraction flask on a 40°C water bath with a jet of clean, dry air to about 30 ml. It is then transferred

[27] Reported values for efficiency of extraction under these conditions range from 85 to 90% for the pesticides (*45*) (*46*) (*48*). It is recommended that each analyst repeatedly check on extraction efficiency.

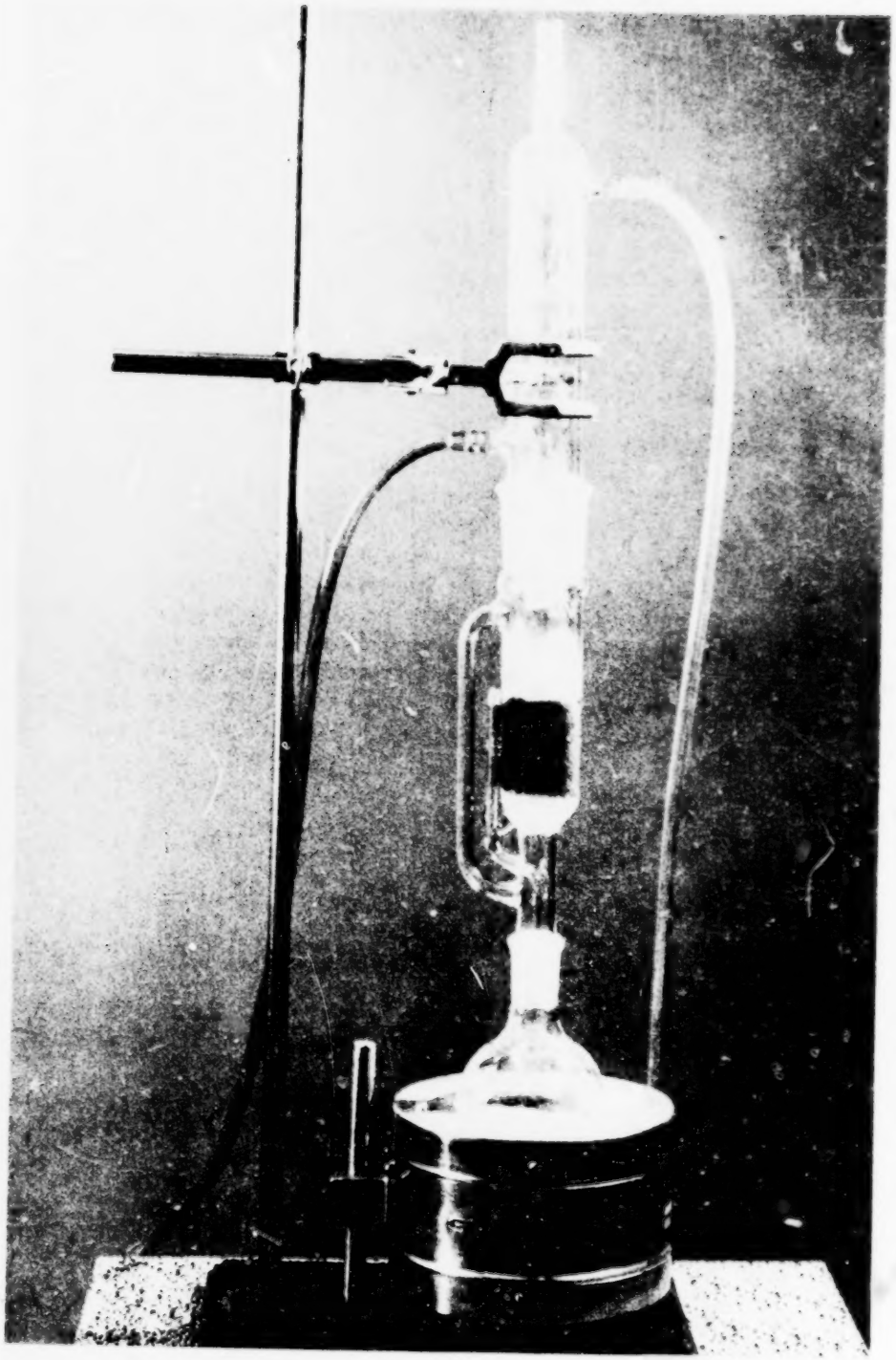

Figure 17. Soxhlet Extraction of Bottom Samples

to a tared 50-ml beaker and evaporated to apparent dryness at room temperature. When possible, the weight of the extract is obtained.

The extracts are cleaned up by column chromatography using the method described in section A.2.b., page 15. The aromatic fraction is evaporated to 5 to 10 ml and transferred to a 15-ml graduated centrifuge tube and evaporated to an appropriate volume for spotting on the TLC plate.

3. Thin Layer Chromatography

The concentrated extract is spotted on a TLC plate and developed in the same manner as the CCE aromatic fractions. The entire extract is spotted whenever possible. However, care must be taken so that the plate is not overloaded. Thus, it may be possible to spot only an aliquot of some samples. Samples high in total organics are in this category.

The four eluted sections are subjected to gas chromatographic analysis. Recoveries of standard pesticides from the thin layer ranging from 85 to 98% are obtainable.[28]

[28] It is recommended that each analyst repeatedly check the TLC recovery efficiency.

IV. DETERMINATIVE STEPS

The identification and measurement of chlorinated hydrocarbons in surface water require extremely sensitive techniques. In small samples the low concentrations which require identification and measurement often provide only a few nanograms (10^{-9} gram) of a pesticide. Carbon adsorption samples usually contain larger amounts for analysis. Gas chromatography, thin layer chromatography, and infrared spectroscopy are employed as corroborative and determinative steps.

A. GAS CHROMATOGRAPHY

Electron capture gas chromatography (ECGC) (*51*) is used for identification and measurement because of its sensitivity. Microcoulometric titration gas chromatography (MCTGC) (*52*), although less sensitive, is specific for halogenated substances. The use of both systems combines the advantages of specificity and sensitivity.[29] Sample gas chromatographic traces for the ECGC and MCTGC systems are included in Appendix Two.

1. Application of Electron Capture Gas Chromatography

a. Extracts of TLC Sections of CCE Aromatics

Pesticides from each sample separated by thin layer chromatography are contained in acetone in four 15-ml, calibrated, Pyrex centrifuge tubes. The volume, usually three to five ml, is reduced by evaporation to 0.5 ml in a water bath at 40°C. with a jet of clean dry air. A 5-μl aliquot is withdrawn from each tube with a 10-μl Hamilton microsyringe and injected into the previously conditioned and stabilized column.[30] Although these conditions are adequate for the concentration range of pesticides found in most samples, in some instances the volume of the TLC extract must be adjusted by evaporation in a 40° C. water bath or by dilution. It is often possible to predict the need for adjustment of injection volume or the total volume on the basis of the TLC result.

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[29] Some other detectors used for determination of chlorinated and/or thiophosphate pesticides are the sodium thermionic (*53*) (*54*), stacked flame (*55*), flame photometric (*56*), emission spectrophotometric (*57*), and the Burchfield microcoulometric titration cell (*58*).

[30] The instruments employed are Perkin-Elmer models 154–L and 811 equipped with a parallel plate E.C. detector, pulser, D.C. power supply, amplifier, and Leeds & Northrup (0–5 mv) recorder. They are operated with pyrex glass or aluminum columns, 4 ft. long x ¼-inch O.D., packed with 5% D.C. 200 silicone on 60/80 mesh acid-washed Chromosorb P at temperatures of 180 to 195°C. The power supply is operated in the pulse mode and the carrier gas is 95% argon—5% methane at a flow rate of 120 ml/min. (nitrogen carrier gas is used for the D.C. mode). The amplifier is usually operated at an attenuation range of 1 and an attenuation of 16 to 64.

After the chromatogram is obtained, it is examined for peaks which possess retention times and peak geometries which match known pesticides (see Appendix Two, Table 2). Since the aromatic fraction has been separated by TLC, the number of possible pesticides in a given injection is reduced. The areas under these peaks are calculated and compared to a standard calibration curve which is prepared by obtaining peak areas from known quantities of the individual pesticides under identical conditions. The peak areas are measured as illustrated in Figure 18 or by use of a planimeter, disc integrator, or electronic digital integrator.

The calibration curve is obtained by plotting peak area in square inches against sample size in nanograms (see Appendix Two). As seen in the discussion of thin layer chromatography, three curves are necessary for TLC II, three curves for TLC III, and four curves for TLC IV. From the calibration curve, the nanograms of each pesticide per TLC section, W, is calculated.

$$\frac{(\mu l \text{ TLC extract})\ (\text{ng determined/injection})}{(\mu l \text{ injected})} = \text{ng/TLC section} = W$$

b. Extracts of TLC Sections from Solvent Extract of Bottled Samples

Essentially the same procedure as outlined in IV.A.1a is employed except for the changes noted herein. The TLC extracts of sections II, III, and IV are reduced in volume to 0.2 ml. A 5-μl aliquot is chromatographed with an attenuation of less than 64.

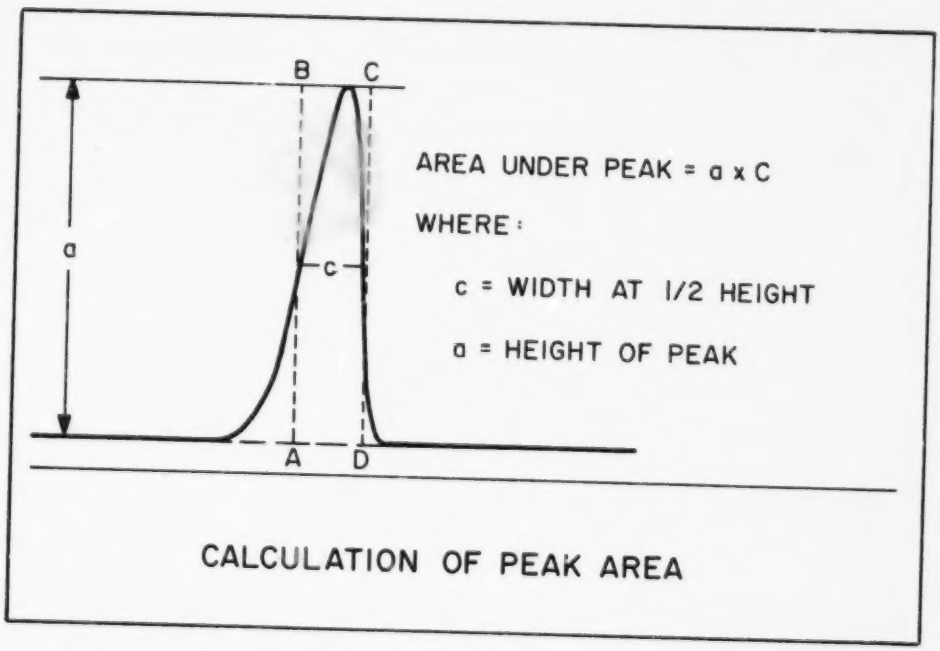


Figure 18. Calculation of Peak Area

The nanograms of each pesticide determined per TLC section, W, is calculated.

$$\frac{(\mu l \text{ TLC extract})\ (\text{ng determined/injection})}{(\mu l \text{ injected})} = \text{ng/TLC section} = W$$

2. Application of Microcoulometric Titration Gas Chromatography

a. Extracts of TLC Sections of CCE-Aromatics

In many instances the entire remainder of the extracts of sections II, III and IV must be injected in order to elicit a response within the sensitivity of the instrument. The result of the ECGC run is used as a guide in the choice of an injection volume. The volume selected is injected into the microcoulometric titration gas chromatographic system.[31] The procedure for identifying and measuring pesticides from the gas chromatographic traces are identical to those described for the ECGC system (see Appendix Two, Table 2). The nanograms per TLC section, W, are calculated as previously.

$$\frac{(\mu l \text{ TLC extract})\ (\text{ng determined/injection})}{(\mu l \text{ injected})} = \text{ng/TLC section} = W$$

b. Extracts of TLC Sections From Solvent Extracts of Bottled Samples

Using the result of the ECGC injection as a guide, an aliquot of extracts II, III, and IV is selected and injected under the same conditions as described for the CCE-aromatic-TLC extract in IV.A.2.a. The evaluation of the chromatographic trace in terms of identification and measurement is carried out as described previously.

$$\frac{(\mu l \text{ TLC extract})\ (\text{ng determined/injection})}{(\mu l \text{ injected})} = \text{ng/TLC section} = W$$

3. Calculations

a. Bottled Samples

$$\frac{(V_{wx})\ (V_t)\ (W_i)}{(V_{sp})\ (V_i)\ (V_{sa})\ (E)} = \mu g \text{ pesticide/liter}$$

V_{wx}	=	volume of solvent representing extract of water sample	(μl)
V_{sp}	=	volume of V_{wx} which was spotted on TLC	(μl)
V_t	=	volume of solvent representing extract of TLC section	(μl)
V_i	=	volume of V_t which was injected	(μl)
V_{sa}	=	volume of water from which extract was made	(ml)
W_i	=	weight of pesticide determined in injection, V_i	(ng)
E	=	efficiency of extraction	(decimal)

[31] The instruments employed are Micro-Tek models 2503R and 179 DSS equipped with a S–100 furnace, T–300 titration cell, and a C–200 coulometer (Dohrmann Inst., Mountain View, California), and a Minneapolis-Honeywell Brown (0–1 mv) recorder equipped with a disc integrator. They are operated with pyrex glass or aluminum columns, 4 ft. long x ¼-inch O.D., packed with 5% DC 200 silicone on 60/80 mesh acid-washed chromosorb P, at a temperature of 200°C, with a carrier gas of helium at a flow rate of 120 ml/min. The injection block temperature is 240°C. The coulometer is usually operated at a sensitivity of 200 to 300 ohms.

(1) If the entire volume of the orginal extract, V_{wx} is spotted on TLC, then $\frac{V_{wx}}{V_{sp}}$ equals 1 and the term is eliminated.

Then, $\frac{(V_t)\ (W_i)}{(V_i)\ (V_{sa})\ (E)} = \mu g$ pesticide/liter

If, in addition, the weight (μg) of pesticide/TLC section, W, has been determined previously:

$$W = \frac{(V_t)\ (W_i)}{V_i}$$

Then the calculation reduces to:

$$\frac{(W)}{(V_{sa})\ (E)} = \mu g \text{ pesticide/liter}$$

b. Carbon Adsorption Samples

$$\frac{(V_{af})\ (V_t)\ (W_i)\ (C_{af})\ (10^{-6})}{(V_{sp})\ (V_i)\ (W_{af})} = \mu g \text{ pesticide/liter}^{32}$$

W_{af}	= weight of aromatic fraction	(mg)
C_{af}	= concentration of aromatic fraction	($\mu g/l$)
V_{af}	= volume of dissolved aromatic fraction	(μl)
V_{sp}	= volume of V_{af} which was spotted on TLC	(μl)
V_t	= volume of solvent representing extract of TLC Section	(μl)
V_i	= volume of V_t which was injected	(μl)
W_i	= weight of pesticide determined in injection, V_i	(ng)

If the weight (ng) of the pesticide/TLC Section, W, has been determined previously,

$$W = \frac{(V_t)\ (W_i)}{(V_i)}$$

then the calculation reduces to:

$$\frac{(W)\ (V_{af})\ (C_{af})\ (10^{-6})}{(V_{sp})\ (W_{af})} = \mu g \text{ pesticide/liter}^{32}$$

4. Column Packings

Glass and aluminum gas chromatographic columns are used for routine analysis of chlorinated pesticides. These columns can be expected to give satisfactory results up to six months. The types of injections made include: pesticide standards, eluates of TLC sections of CCE aromatic fractions from raw and finished waters, and solvent extracts of grab samples of raw and finished water. Other column packings

[32] In the light of the unknown efficiency of adsorption on and desorption from carbon for most organic compounds, these concentrations must be considered minimum; the actual concentration being equal to or most likely greater than that determined.

have been employed in our laboratory. Some additional column packings used for pesticide analysis are shown in Appendix Two, Table 3. It is recommended that several different column packings be employed for corroborative qualitative identification.

5. Column Conditioning

To obtain optimum response and peak resolution, gas chromatographic columns must be adequately conditioned. Conditioning requirements vary for different column packings and for different pesticides.

Columns packed with acid washed Chromosorb P (60-80 mesh) coated with 5% DC 200 silicone are conditioned, in our laboratory, according to the following procedure. The column is installed in the oven, the carrier gas is adjusted to the proper flow rate, and the column temperature increased very slowly to 200°C. (programmed at a rate of 1 °C. per min.).

It is held at 200°C. overnight and then increased to 225°C. for two to three hours and brought back to 200°C. The column is held at this temperature and 50 to 100 μg quantities of standard pesticides and normal aliquots of actual samples are periodically injected. Conditioning for the electron capture gas chromatograph may require up to three days. Approximately seven days may be required for the microcoulometric gas chromatograph. For some pesticides, such as endrin, the column may require additional conditioning.

Columns tend to lose their response and resolution abruptly and new columns should be pre-conditioned and ready for use when this occurs. Insertion of quartz wool ahead of the column will tend to lengthen the life of the column by retaining the nonvolatile waxes and oils present in many of the samples. Insertion of a Pyrex glass or quartz tube in the injection block is also helpful in extending column life.

B. INFRARED SPECTROPHOTOMETRY

The aromatic fraction of each carbon adsorption sample is prepared for infrared spectroscopy (*13*). A portion of the aromatic fraction is diluted with chloroform. A suitable volume is spread evenly on a simple salt plate. The solvent is evaporated under a heat lamp. A standard 12-minute scan is made on a Perkin-Elmer 137-B Infracord[33]. The resulting spectrum is examined for specific absorption bands coincident with those appearing on standard spectra (see Appendix Three). When pesticides are present in overriding concentrations, spectra are specific enough to permit unilateral identification. In many cases the number and position of the absorption peaks in the spectrum will lend support to the identifications made by previously described chromatography (see Figures 9 and 10).

When sufficient extract is obtained from a bottled water or bottom sample, it is also examined by infrared as described above.

[33] More refined I.R. instruments, equipped with a beam condenser and scale expansion, will provide much greater sensitivity.

V. CONTROL OF INTERFERENCES

When using ultrasensitive analytical techniques, particularly electron capture gas chromatography for pesticide analyses, possible interferences from solvents, activated carbon, and other reagents and materials employed throughout the procedure must be given continuous attention. Adequate steps must be taken to eliminate or minimize any interferences and ensure that, if present, they are taken into consideration in the interpretations of the gas chromatograms.

To accomplish these objectives, all solvents, carbon and other materials are checked routinely by subjecting them to analyses identical to those used for samples.

A. SOLVENT INTERFERENCES

1. Chloroform

All chloroform (analytical reagent grade) used for extraction of the carbon adsorption samples is distilled before use. To determine interferences, a volume of $CHCl_3$ equivalent to that used for extraction of the carbon is distilled a second time. The residue obtained, representing that attributable to $CHCl_3$ which would have been contained in the CCE sample is given the column chromatographic separation [see Section III.A.2a(2)]. One-tenth of the aromatic fraction thus obtained is given the usual TLC cleanup. A volume of the eluate from each section, equivalent to the volume of sample routinely used, is injected into both the electron capture and the microcoulometric gas chromatographs. The significance of interferences, if present, is noted in terms of retention time, peak geometry and peak intensity, i.e., area and height. Interferences, if noted under these conditions, can be considered maximum.

2. Hexane-Benzene

Chromatographic grade hexane and benzene are checked individually as below and, if necessary, distilled in an all-glass system before use in extraction of water grab samples. A volume of hexane-benzene (50 ml, 9:1) or hexane (300 ml) equivalent to that used in the extraction is evaporated to the appropriate volume as described in Section III,B,2. This residue is given the TLC cleanup and the eluates from the four sections are injected into the gas chromatograph as discussed previously.

Other solvents or solvent combinations (ethyl ether, petroleum ether, benzene, acetone, chloroform, and carbon tetrachloride) used for extraction are checked in the same manner.

3. Hexane-Acetone

A volume of hexane-acetone (200 ml, 9:1) equivalent to that used in the extraction of a bottom sample is evaporated as described in section III, B,1,b. The residue is examined by TLC and gas chromatography.

4. Carbon Tetrachloride and Acetone

A quantity of carbon tetrachloride and acetone approximately equivalent to that used for development of the TLC plates and elution of the pesticides from the silica gel are also checked for interferences.

B. CARBON INTERFERENCES

As discussed in Section II entitled Sample Collection, it is possible for small amounts of organic substances to become adsorbed upon carbon in the period between its activation and its use in the cartridge. Together with a precautionary program to reduce the probability of such contamination occurring during transport or storage, an aliquot of the unused carbon is analyzed by a procedure identical to that used for carbon adsorption samples.

1. Carbon Blank

The presence or absence of interferences in the carbon blank are determined according to the procedure for determining the CCE (Section III.A.1.). A quantity of carbon equivalent to that used in a carbon adsorption cartridge is extracted with three liters of double distilled analytical reagent grade chloroform. A blank determination is made whenever a new container is opened. All cartridges filled with carbon from a given container are so recorded. The residue is subjected to the standard column chromatographic separation. One-tenth of the aromatic fraction is given the normal TLC cleanup and gas chromatographic analysis as previously described.

Interferences, if noted under these conditions, would be at maximum effect.

C. OTHER SOURCES OF INTERFERENCE

The silica gel (Davison Code 950) used for the column chromatographic separation, silica gel G used for TLC cleanup, and the anhydrous sodium sulfate used for drying solvent extracts are examined for interferences using the appropriate solvents for elution and development. The eluates are subjected to gas chromatography as previously described. When quantities of residue permit, infrared spectra are determined for solvents, carbon, and other reagents.

The following techniques are suggested for pretreatment and clean-up of the adsorbents used for column and thin layer chromatography. Silica gel (Davison) may

be washed with acetone-ether (1:1) followed by ether, then air dried, and finally heated at 110-120°C. overnight (*59*). Pretreatment of florisil may include washing with acid, alkali, absolute methanol, and distilled water followed by heating at 650°C. for 1-3 hours. Spent florisil may be recovered by washing with diethyl ether, benzene, ethanol, and distilled water then heating overnight at 130°C. (*60*). Silica gel G and alumina TLC plates may be washed by developing with distilled water and drying at 75°C. for 15 minutes. The washing is repeated and the plate is dried at 75°C. for 30 minutes (*61*). Alternately, 50% aqueous acetone may be used for washing. The plate is air dried for 5 minutes and then heated at 80°C for 45 minutes (*62*). The adsorbents prepared by these techniques may be stored in a desiccator from 4 to 7 days without significant loss of activity.

Glassware may also be a source of contamination. Therefore all glassware is cleaned up as described in Section II.B.3. If the type and size of the glassware permits, it is heated in a muffle furnace at about 400°C. for 15 to 30 minutes.

D. INTERPRETATION

The interpretation of all gas chromatographic analyses are made in light of any interferences determined by the foregoing procedures. If interferences are present and are significant enough to invalidate specific results, either qualitatively or quantitatively, these results are discarded.

VI. SENSITIVITY AND SPECIFICITY

A. SENSITIVITY

In discussing sensitivity, in terms of concentration, it must be pointed out that concentrations obtained with the carbon adsorption method are minimum values (see Section IV).

1. Carbon Adsorption Extracts Examined by Electron Capture Gas Chromatography

The electron capture detector varies in its sensitivity for the various members of the chlorinated hydrocarbon pesticide series. However, it is, in general, capable of detecting absolute quantities of 1 nanogram (10^{-9} g) or less on a routine basis. Remembering that (a) only 1/10 of the aromatic fraction is subjected to TLC and (b) that only 1/1000 of the TLC section extract is chromatographed, then under these conditions a peak calculated to represent 1 nanogram is equivalent to:

$$1 \text{ ng} \times 10 \times 100 = 1{,}000 \text{ ng} = 1 \ \mu\text{g}$$

If a sample had a measured volume of 5,000 gallons (approximately 20,000 liters) the lowest detectable concentration can be estimated at 1 μg/20,000 liters or 0.00005 μg/1. However, if it is also recalled that (c) only a portion (variable) of the CCE is separated by solubility to obtain the neutral fraction and (d) that only a portion of the neutral fraction is separated on the silica gel column to obtain the aromatic fraction, the lowest detectable concentration is greater than 0.00005 μg/1. Since the values for (c) and (d) vary, the lowest detectable concentration, under these conditions, has been estimated conservatively at 0.001 μg/1. Samples measuring less than 5,000 gallons induce a corresponding increase in the estimate of the lowest detectable concentration. It must be noted, however, that the electron capture detector sensitivity can often be used to detect quantities of less than 1 nanogram for some pesticides if background interference is negligible.

In the event (a) it is necessary to detect concentrations of less than 0.001 μg/1, (b) the additional effort is justified, and (c) interferences are negligible, the use of all of the CCE, all of the neutral fraction, all of the aromatic fraction, and all of the TLC section extract has a potential for detecting a concentration of 0.00000005 μg/1 in a 5,000-gallon sample and 0.00000025 μg/1 in a 1,000-gallon sample.

2. Carbon Adsorption Extract Examined by Microcoulometric Titration Gas Chromatography

Since 2-10 ng are required to produce a minimum recognizable response for most chlorinated hydrocarbon pesticides and recalling that it is usually necessary to inject all of the TLC section extract (rather than 1/100 as in the case of ECGC) the lowest detectable concentration under these procedures has been conservatively estimated at 0.001 μg/1. Potentially, using the entire sample and with significant additional effort, detection of 0.0000025 μg/1 is possible.

3. Bottled Sample Extracts Examined by Electron Capture Gas Chromatography

The lowest measurable concentration is approximately 0.001 μg/1 in a 1-liter water sample and 0.001 μg/100 g or 0.010 μg/Kg in a bottom sample.

4. Bottled Sample Extracts Examined by Microcoulometric Titration Gas Chromatography

The lowest measurable concentration is 0.002-0.010 μg/1 in a 1-liter water sample and 0.002-0.010 μg/100 g or 0.02-0.10 μg/Kg in a bottom sample.

B. SPECIFICITY

1. Carbon Adsorption Samples

In the examination of CCE for chlorinated hydrocarbon pesticides by the procedure outlined, it is demonstrated that the pesticide: 1—is adsorbed on carbon, 2—is desorbed with chloroform, 3—is ether soluble, 4—is not water soluble, 5—is not acidic, 6—is not basic, 7—is neutral, 8—is benzene soluble, 9—moves on TLC in the same fashion as a given standard, 10—is eluted from ECGC at the same retention time as, and having the same peak geometry as a given standard, 11—is identical to the same standard when chromatographed with MCTGC in terms of its retention time, peak geometry, and degree of chlorination, and produces an infrared spectrum which in many cases supports the identifications made by chromatography.

2. Bottled Samples

The examination of bottled samples by the procedure outlined provides for three corroborative chromatographic identifications which serve as a three-way cross check on identification.

APPENDIX ONE

ENGINEERING ASPECTS OF SAMPLING BY THE CARBON ADSORPTION METHOD

The carbon adsorption method of organics sampling consists of the passage of up to 5,000 gallons of *raw water* at rates up to ½ gallon per minute through a carbon adsorption column. Following the sample run, the column is shipped to the laboratories in Cincinnati, Ohio, for analysis.

I. TYPES OF SAMPLING EQUIPMENT IN USE

A. GENERAL

At the present time there are three types of carbon adsorption sampling apparatus used in the Water Pollution Surveillance System. The first and oldest of these consists of a piping arrangement that was originally assembled and installed at the sampling location. This device is referred to as the manual type installation and is discussed on page 41. The second type is a prefabricated system with automatic backwash of a sand prefilter. Two models of this type system were developed for extensive use in water pollution surveillance. One model is a panel unit equipped with automatic backwash device, designed for mounting on a wall inside a building.

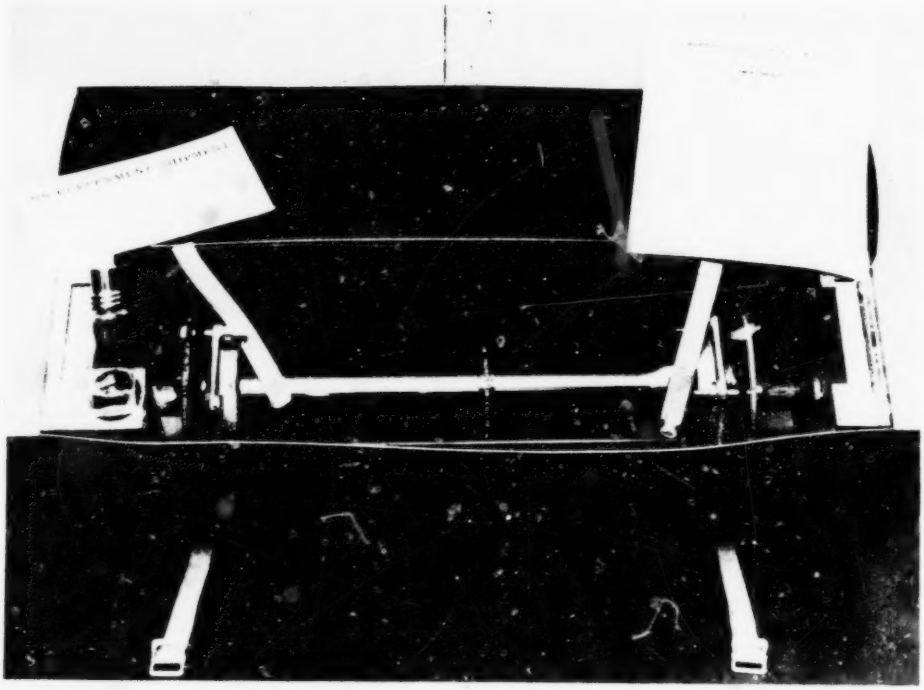


Figure 19. Carbon Adsorption Column and Shipping Container

A second model is similar to the panel unit, but is built into a protective housing for operation in remote or outside the plant use. The third and newest type of sampler utilizes a low flow rate for more efficient collection of organic substances. Two models of this type of sampling system have been designed and recently placed in use following successful field evaluation.

B. DESCRIPTION OF CARBON ADSORPTION COLUMN (CAC)

The CAC consists of a piece of Pyrex glass pipe 3 inches in diameter and 18 inches long. The ends are fitted with brass plates and $^3/_4$-inch galvanized nipples. A stainless steel screen is fixed in a neoprene gasket at both ends. The filter unit arrives at the station packed with activated carbon ready for use. A special shipping container is provided for returning the filter. The unit, with the shipping container, is shown in Figure 19. A modified cartridge with a hose type connection has been designed for use with the low flow rate sampling equipment.

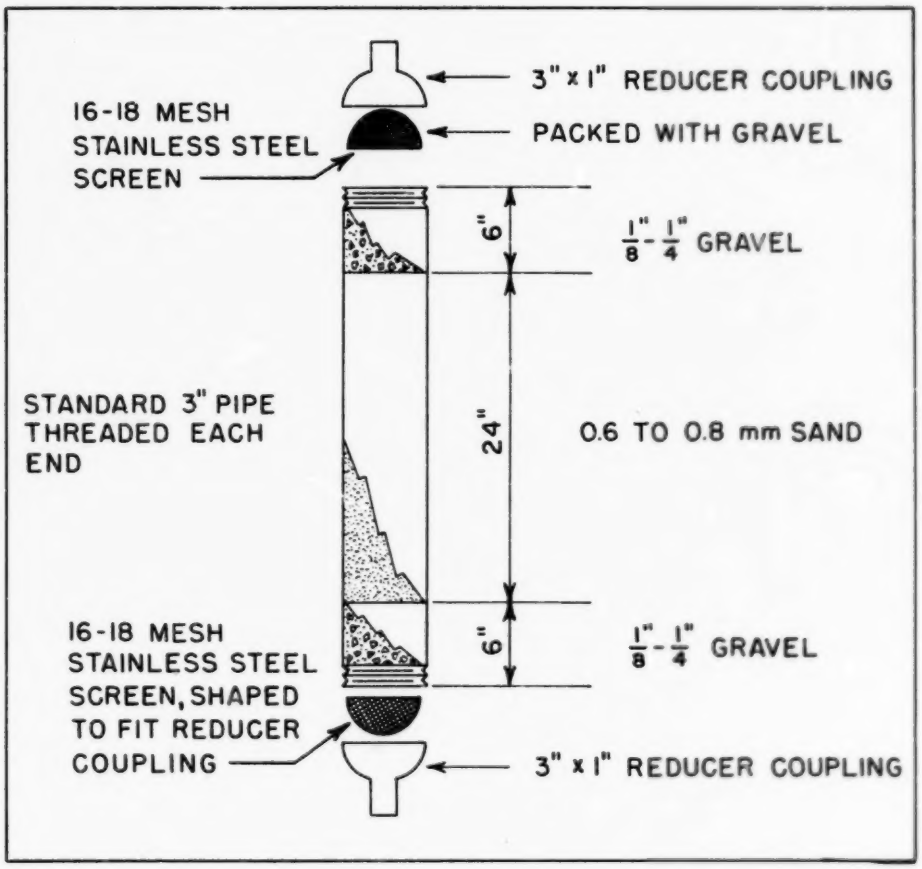

3" x 1" REDUCER COUPLING
16-18 MESH STAINLESS STEEL SCREEN
PACKED WITH GRAVEL
6"
1/8" - 1/4" GRAVEL
STANDARD 3" PIPE THREADED EACH END
24"
0.6 TO 0.8 mm SAND
16-18 MESH STAINLESS STEEL SCREEN, SHAPED TO FIT REDUCER COUPLING
6"
1/8" - 1/4" GRAVEL
3" x 1" REDUCER COUPLING

Figure 20. Details of Sand Prefilter

C. PRESETTLING AND PREFILTERING

Turbid river waters frequently clog the CAC when attempting to sample 5000 gallons. To permit this amount of water to pass through the column a presettling tank and prefilter containing sand and gravel were sometimes required for the manual and automatic backwash sampling systems designed for use at 0.25 to 0.50 gpm flow rates. This equipment has not been required for satisfactory operation of the newer low flow rate designs. A standard hot water tank connected with the inlet at the bottom and outlet at the top and with a clean-out tap at the bottom can serve as a presettling tank. The outlet is connected to the prefilter containing sand and gravel. The tank must be flushed at intervals to prevent accumulation of solids.

The sand prefilter consists of a steel pipe 3 feet long and 3 inches in diameter, threaded at both ends, and equipped with 3 by 1-inch reducer couplings. Two cupped stainless steel screens are fitted to the reducer couplings. The space between the screens is packed with gravel and sand as shown in Figure 20.

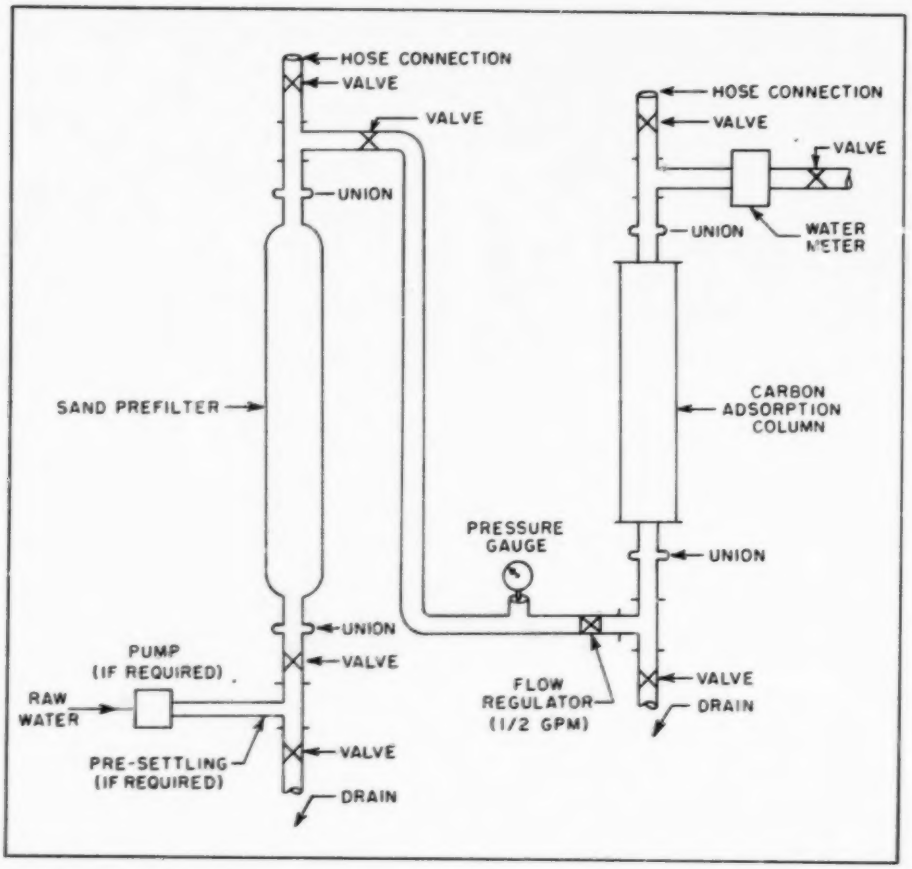


Figure 21. Schematic Diagram of an Installation with Manual Backwash

D. INSTALLATIONS WITH MANUAL BACKWASH

The presettling tank, the sand prefilter and the CAC are installed at the most convenient source of raw water. If less than 15 psi pressure is available, it may be necessary to pump the water through the system. A drawing of a workable system is shown in Figure 21.

A water meter located at the end of the system is used to measure the volume of water sampled. This is usually a disc-type meter, or oscillating piston-type meter, registering in gallons and capable of measuring flows as low as ¼ gallon per minute. If necessary, a ½-gpm flow regulator or a valve following the meter can be used to control the flow rate.

Fine carbon dust washes out of the CAC when it is first started. A few gallons of water are passed through the top connection and through the CAC drain before the meter is cut in, to keep the meter free of the carbon.

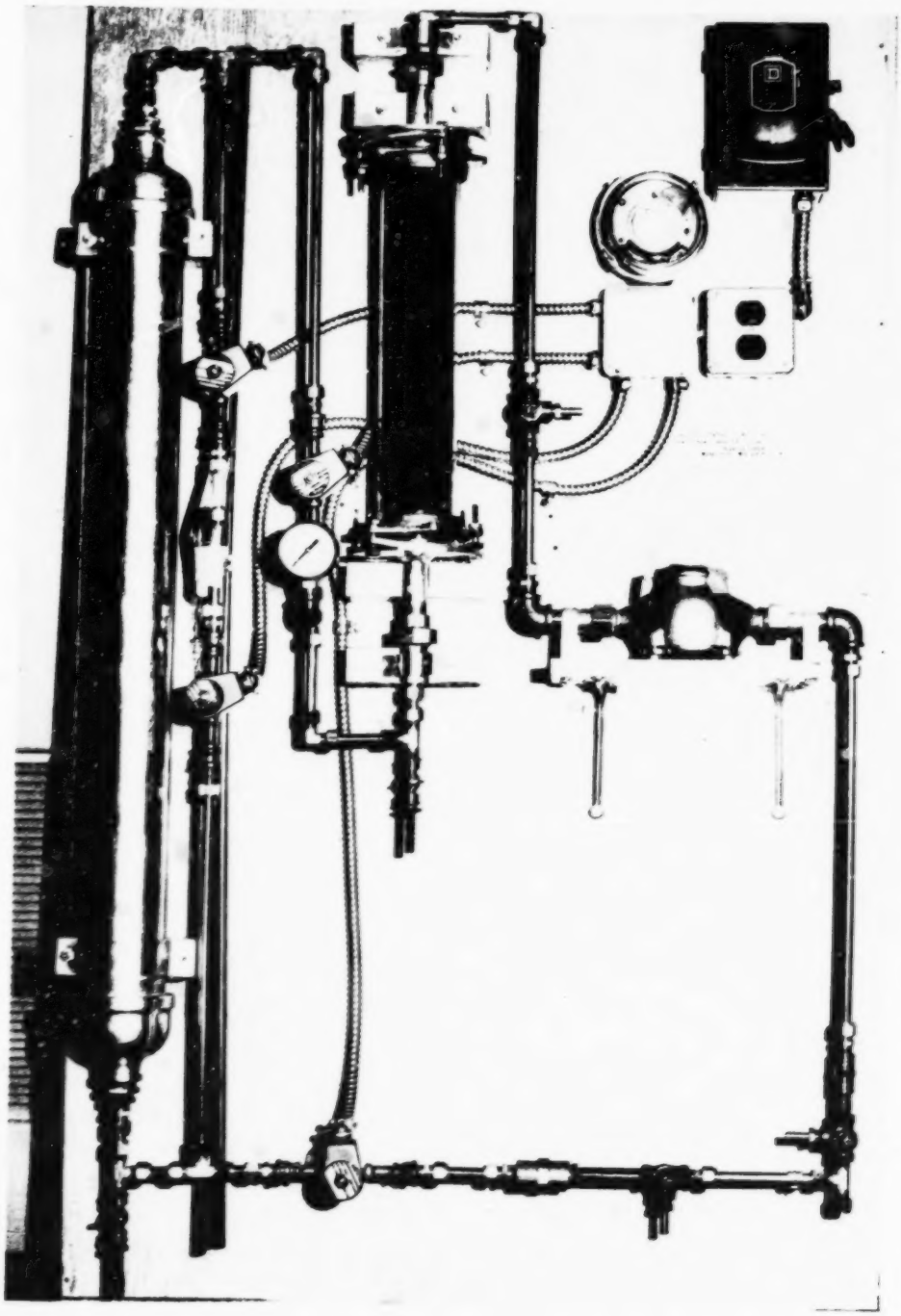


Figure 22. Carbon Adsorption Unit Model H_2O–M1C with Sand Prefilter and Automatic Backwash

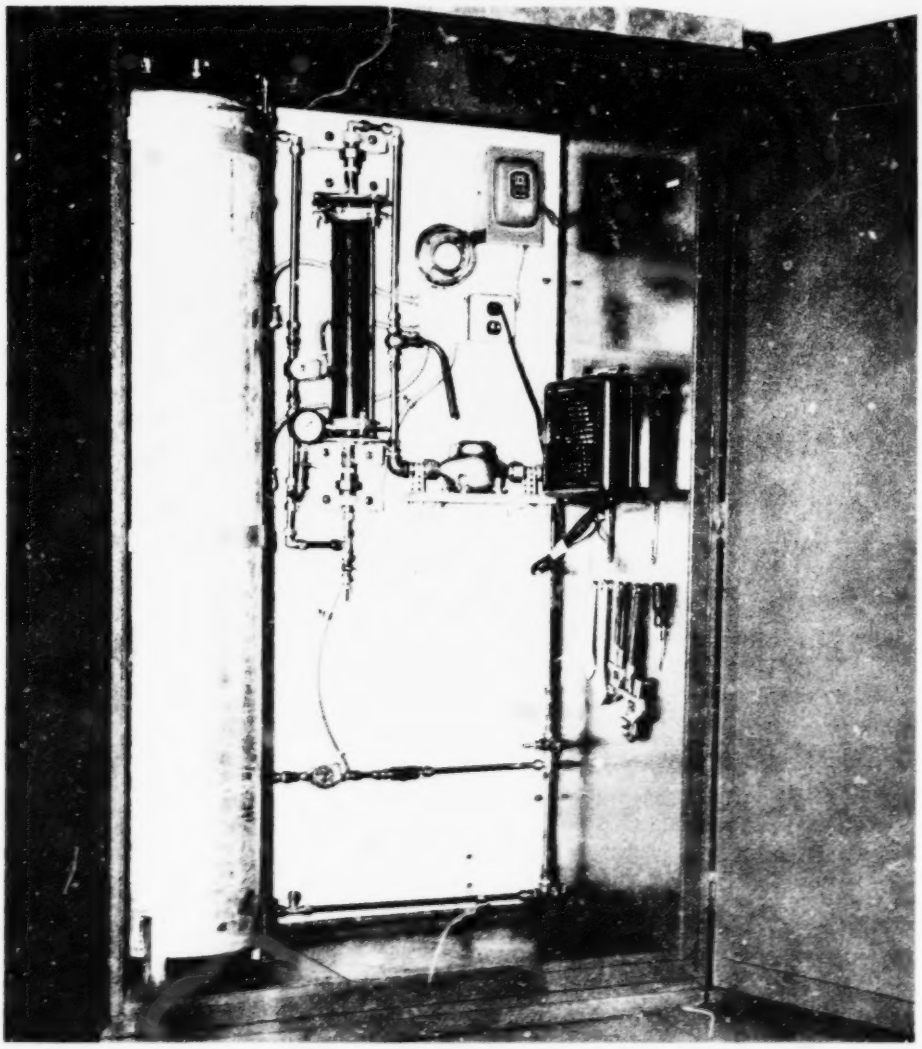


Figure 23. Carbon Adsorption Unit Model H_2O–M2C with Presettling Tank and Auxiliary Equipment in Shelter

E. INSTALLATIONS WITH AUTOMATIC BACKWASH

Preassembled panel units with automatic backwash of the sand prefilter were developed to ease installation and operation of the organics sampling apparatus. Figure 22 shows the Model H_2O-M1C panel unit designed for installation in water treatment plants and other buildings. This equipment has an electric timer and solenoid valves to backwash automatically the sand prefilter. The panel includes an electric

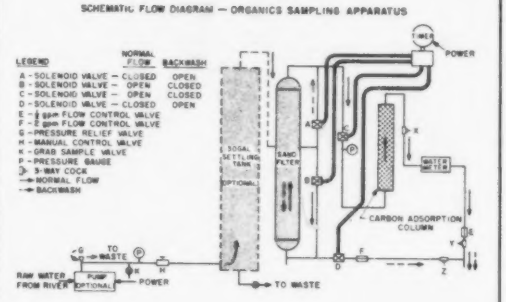
Organics Sampler
Models H_2O – M1C and H_2O – M2C

A) Sample Type - Organics

B) Sampling Volume - 5000 Gallons

C) Sampling Frequency - Monthly

D) Information - U.S. DEPARTMENT OF INTERIOR
Federal Water Pollution Control Administration
Water Quality Activities
1014 Broadway
Cincinnati 2, Ohio



SAMPLING PROCEDURE

1) Install carbon filter

2) Flush carbon filter, in place through cock "X", with raw river water to remove carbon fines.

3) Begin sampling run and record date and initial water meter reading on carbon filter log sheet

4) Record daily, if possible, the water meter readings on the carbon filter log sheet until the required volume of raw water has been sampled. (Approximately 7 days)

5) After filtering required volume record removal date, water meter reading and return carbon filter and log sheet to Cincinnati, Ohio within the provided shipping container

6) Upon receipt of used carbon filter in Cincinnati, a new filter will be returned for next sampling run

Note: In highly turbid waters, the filter may tend to clog, turning filter end to end and/or backwashing for 2 to 3 minutes may be used once to obtain at least a 2000 gallon sample

Figure 24. Schematic Flow Diagram with Sampling Procedure for Organic Sampler Models H_2O–M1C and H_2O–M2C

disconnect switch of fuse-plug-type and grounding-type duplex outlet for pump. It also has three 3-way cocks, one to protect the water meter from fine carbon at the beginning of sampling, one to facilitate checking of the flow control valve and water meter, and one to check backwash performance.

For remote locations the sampling apparatus is installed in an insulated equipment shelter. An organic sampling field unit, Model H_2O-M2C, containing preassembled panel apparatus, a 30-gallon presettling tank, electric space heater, and auxiliary equipment is shown in Figure 23. The pumping system will vary depending on the needs of the individual sampling station. A submersible pump was used for the field unit shown in Figure 23. The equipment shelter has space for a jet centrifugal-type pump, or other acceptable motor pump unit.

A prefabricated metal building may be provided, where required, to provide a permanent shelter for equipment and operating personnel. This type of building is usually installed on a reinforced concrete base. An organics-sampling panel unit (Model H_2O-M1C), a pumping system, and other sampling equipment can be installed in this type of facility.

Figure 24 shows the schematic flow diagram with sampling procedure for the organics sampler. (Models H_2O-M1C and H_2O-M2C.)

F. LOW FLOW RATE ORGANICS SAMPLING

Studies of optimum sampling rate and sample volume for maximum recov-

ery of organics by the standard carbon adsorption method (9) showed that sampling efficiency can be increased by the use of smaller volumes and lower flow rates. Castelli and Booth (*30*) designed a practical system to control flow of raw water through the carbon column at low rates and measure the throughput. This system was developed further by Reid and Stierli (*31*) for field evaluation.

1. Comparative Field Tests

A preliminary field evaluation of two low flow rate samplers in comparison with conventional sampling apparatus was conducted at the Surveillance System Field Test Station on the Little Miami River, Cincinnati, Ohio, during February and March 1964. Four sampling panel units were operated in parallel, two conventional units at the rate of ½-gpm flow rate and two prototype samplers at the reduced rate of 100 ml/min, or less. Sample volumes for the higher flow rate were approximately 5000 and 1000 gallons and about 250 gallons (1000 liters) for the lower rates. Figure 25 shows the apparatus as it was installed at the Field Test Station. The panels in the foreground and left background operated at ½ gpm, while the other two panels (one behind the center panel and the other in the right background) operated at the reduced rates of flow.

Included in the study were tests with and without presettling and sand prefiltering. The performance of the low flow rate equipment was satisfactory without presettling and prefiltering even though turbidities of up to 1750 Jcu were measured for the raw water.

The study indicated that approximately double the amount of total organic materials is recovered per gallon of water passing through the "regular"[34] carbon column by reducing the throughput from 5000 to 1000 gallons. Approximately five times the amount of total organic substances was recovered per gallon of water by decreasing the rate flow from ½ gpm to 100 ml/min and reducing the throughput from 5000 gallons to approximately 1000 liters. The use of "all fines"[35] carbon columns further increased the recovery of organic materials when sampling at conventional and low flow rates.

The comparative tests with and without presettling and prefiltering indicate significantly greater recovery of organic materials from raw water samples receiving no processing prior to flow through the carbon column. Six to eight times the amount of total organic substances per gallon of water were recovered from "all fines" carbon columns operated with low flow rates and no preprocessing of turbid water as compared with parallel "regular" carbon columns receiving presettled and prefiltered water at a flow rate of ½ gpm and a 5000-gallon throughput. The filtering action of either "all fines" or "regular" carbon columns can be utilized in the low flow rate samplers to obtain an organics sample which includes much of the silt and other particulate matter transported by a river. This is of special importance for measurement of pesticides in water as the transported material may carry specific substances of concern.

Additional field tests of low flow rate samplers were conducted on the Mis-

[34] The "regular" carbon column is packed with two types of carbon (see page 10.)
[35] The "all fines" carbon column is packed only with 30 mesh Nuchar C–190 carbon. (See page 10.)

souri River at Omaha, Nebraska, the Arkansas River at Little Rock, Arkansas, the Columbia River at Pasco, Washington, and the Escambia River at Century, Florida. Over 30 production model low flow rate samplers were installed during 1965 for water pollution surveillance.

2. Low Flow Rate Organics Sampler, Model LF-1

Figure 26 shows a Model LF-1 organics sampler for water. The carbon column and accessory equipment is assembled on a plywood panel 2'6" wide by 3' high.

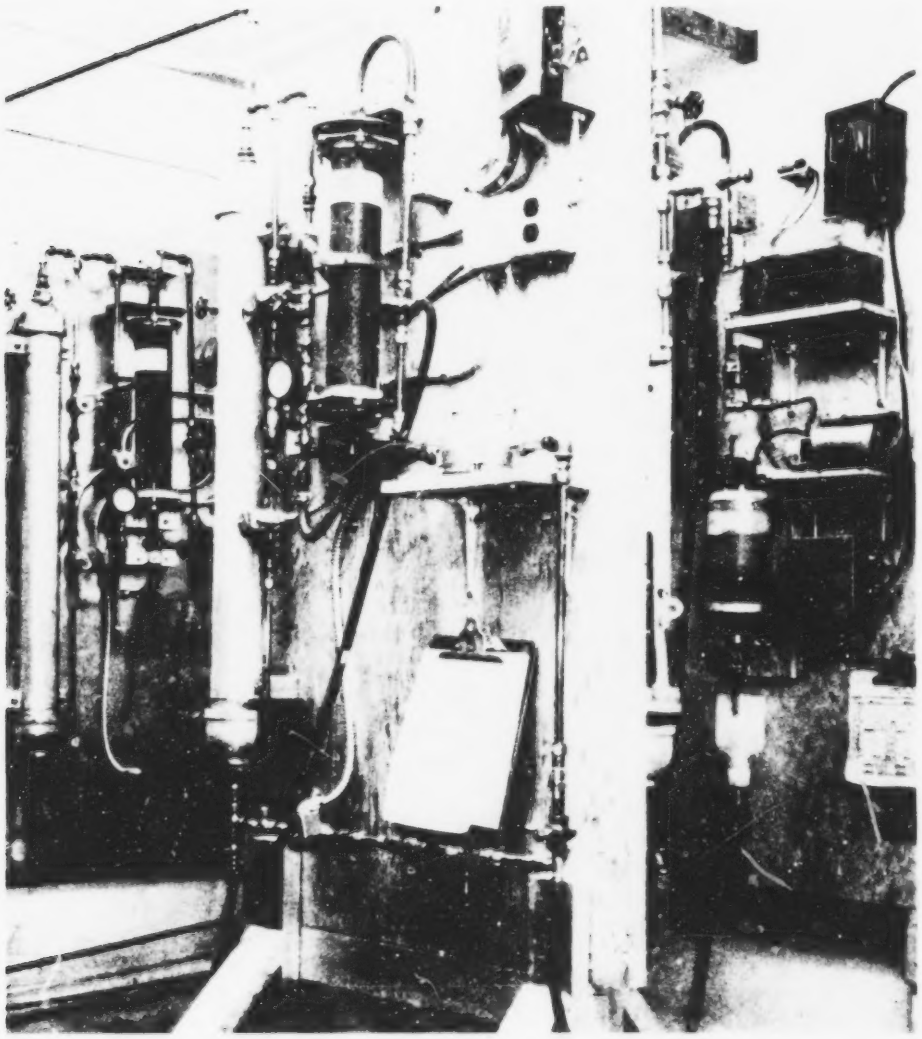


Figure 25. Equipment Installed in Field Test Station for Field Evaluation of Low Flow Rate Samples in Comparison with Conventional Sampling Apparatus

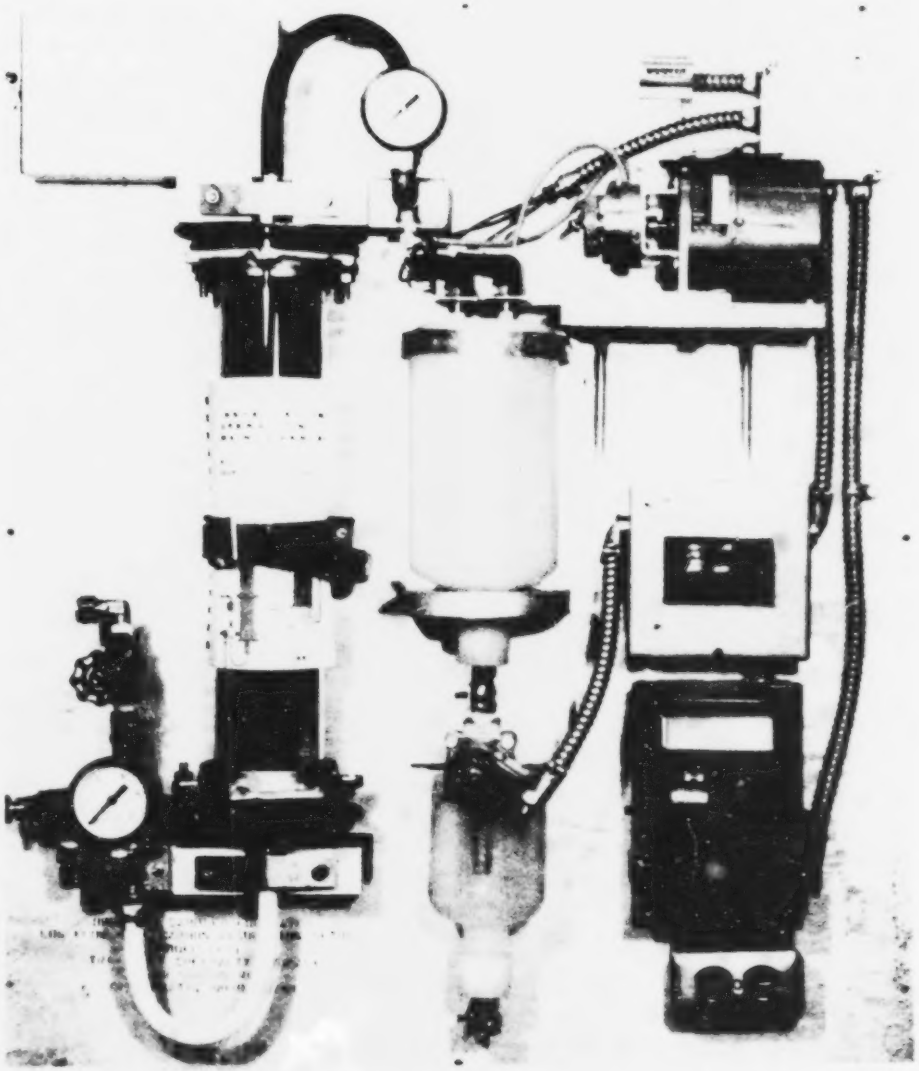


Figure 26. Low Flow Rate Organics Sampler, Model LF-1

Raw water enters the sampling system at the left and passes through a 1-gpm flow control valve located behind the pressure gauge. An adjustable pressure relief valve to the left of the pressure gauge is used to control the pressure for operation within 3 to 15 psi. The sample water passes through a Teflon tube to the carbon column. After passing up through this column, the water flows through a rubber hose to a peristaltic action type pump for control of flow at approximately 100 ml/min.

The water goes from the pump to the volumetric measuring tank which con-

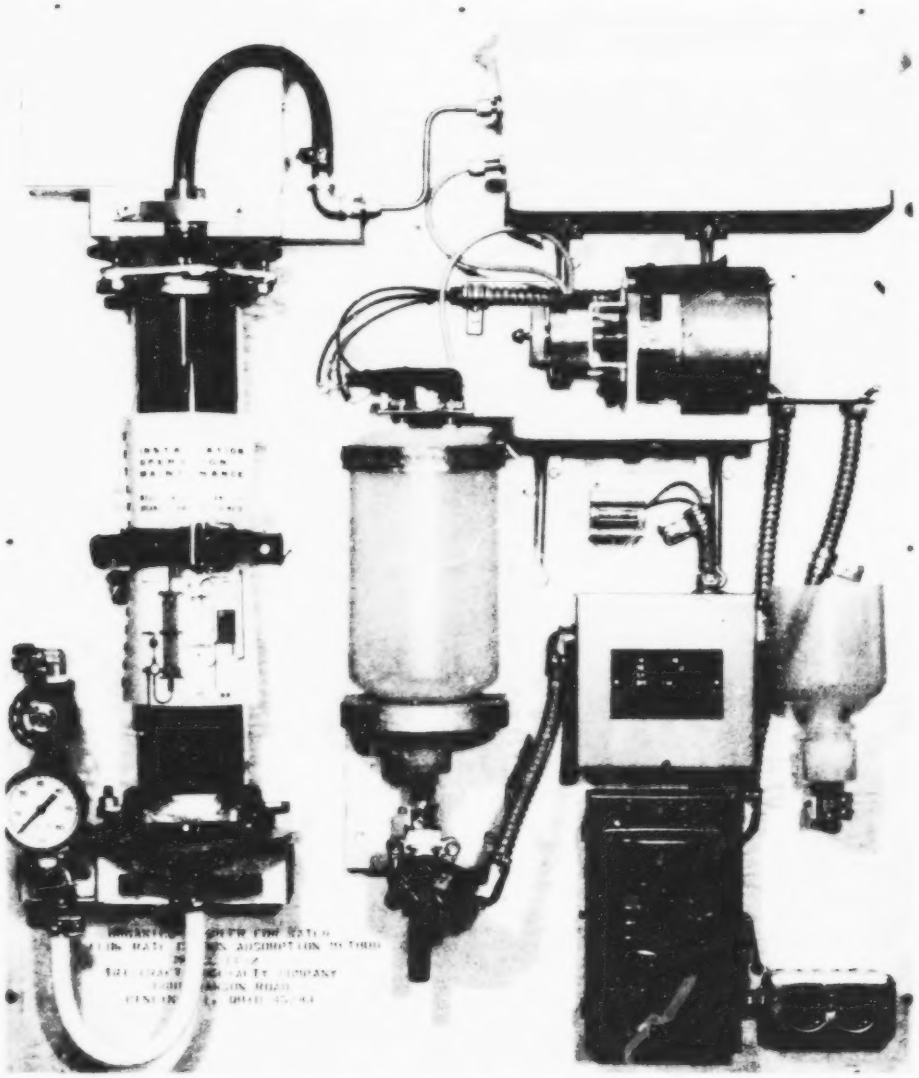


Figure 27. Low Flow Rate Organics Sampler, Model LF 2

3. Low Flow Rate Organics Sampler, Model LF-2

A Model LF-2 organics sampler is shown on Figure 27. This sampler is similar to the Model LF-1 sampler except it includes a constant head tank between the carbon column and peristaltic action type pump. A float in the constant head tank controls the flow of water from the carbon column. The flow through the constant head tank is regulated by the pump during operation.

The volumetric tank, liquid level control, solenoid valve, digital counter and fused electric disconnect switch operate similarly on Models LF-1 and LF-2.

Satisfactory operation can be obtained with the Model LF-2 sampler with pressures ranging from 5 to 50 psi. The constant head tank enables the peristaltic action type pump to operate with a minimal variation in flow rate. However, flow rates and throughputs for the Model LF-2 are from 10 to 25% lower than Model LF-1 for parallel sampling.

II. PUMPING SYSTEM

Pumps, piping, and accessories are selected to suit the specific conditions of each station. Shallow and deep well-type jet centrifugal pump systems are in use at many stations to bring raw water from a representative sampling point to the sampling apparatus. Submersible pumps with helical screw rotors and synthetic rubber stators, rotary pumps with flexible impellers, and other pumping mechanisms may be installed to meet individual needs.

It is important that the pump does not contaminate the sample through grease-type packing or other sources. The pump must have a greaseless-type rotary shaft seal or special packing material to avoid contamination. Laboratory control procedures are employed to assure that the pump does not contaminate the sample. New pumps are sometimes grease-coated and must be thoroughly cleaned before being put into service. Piping strainers, check valves, and all other accessories that come in contact with the raw water pumped to the CAC filter are also cleaned. (See Precautions, paragraph below.)

III. PRECAUTIONS

The purpose of the CAC is to adsorb small amounts of organic impurities from the water in as great a quantity as possible. *It is important to avoid contamination of the carbon from other organic sources.* Hence the following precautions are observed:

A. New strainers, pipe fittings, and other accessories are usually coated with oil or grease. The oil is removed by washing in kerosene or chloroform followed by a detergent wash before fittings are used for making connection to the CAC.

B. Ordinary organic pipe jointing compounds are *not* used. Red lead (lead oxide) mixed to a paste with water can be used for this purpose.

C. Except as noted below, *plastic* hose is avoided, and if rubber hose is used in any connections it is flushed thoroughly before being connected to the CAC. Copper tubing is ideal for connections. *NOTE:* Polyethylene pipe and PVC (polyvinyl chloride) pipe meeting National Sanitation Foundation (NSF) standards for drinking water use are acceptable. Teflon hose also is satisfactory for use.

IV. USE OF CARBON COLUMN DATA SHEET

Accurate flow measurements are important. A sample data sheet is used to record flow and other pertinent information.

APPENDIX TWO

CHROMATOGRAMS, SAMPLE CALIBRATION CURVES, INFRARED SPECTRA, AND STRUCTURAL FORMULAE

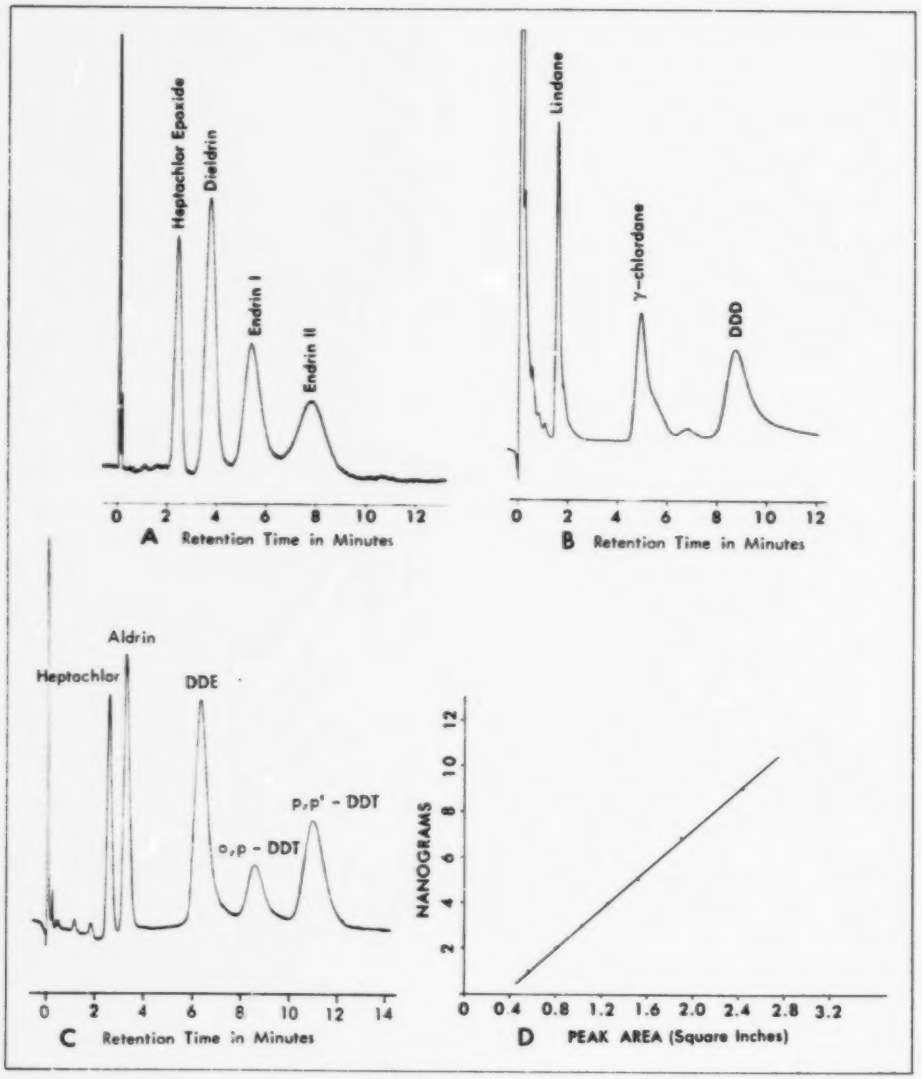


Figure 28

EC Gas Chromatograms of Standard Pesticides in (A) TLC Section II (heptachlor epoxide—1 ng, dieldrin—2 ng, endrin—4 ng), (B) TLC Section III (lindane—1 ng, γ-chlordane—2 ng, DDD—3 ng), (C) TLC Section IV (heptachlor—0.5 ng, aldrin—0.5 ng, DDE—1 ng, DDT—2 ng). (D) Sample Calibration Curve for Dieldrin (ECGC). See page 28, footnote 30 for operating conditions.

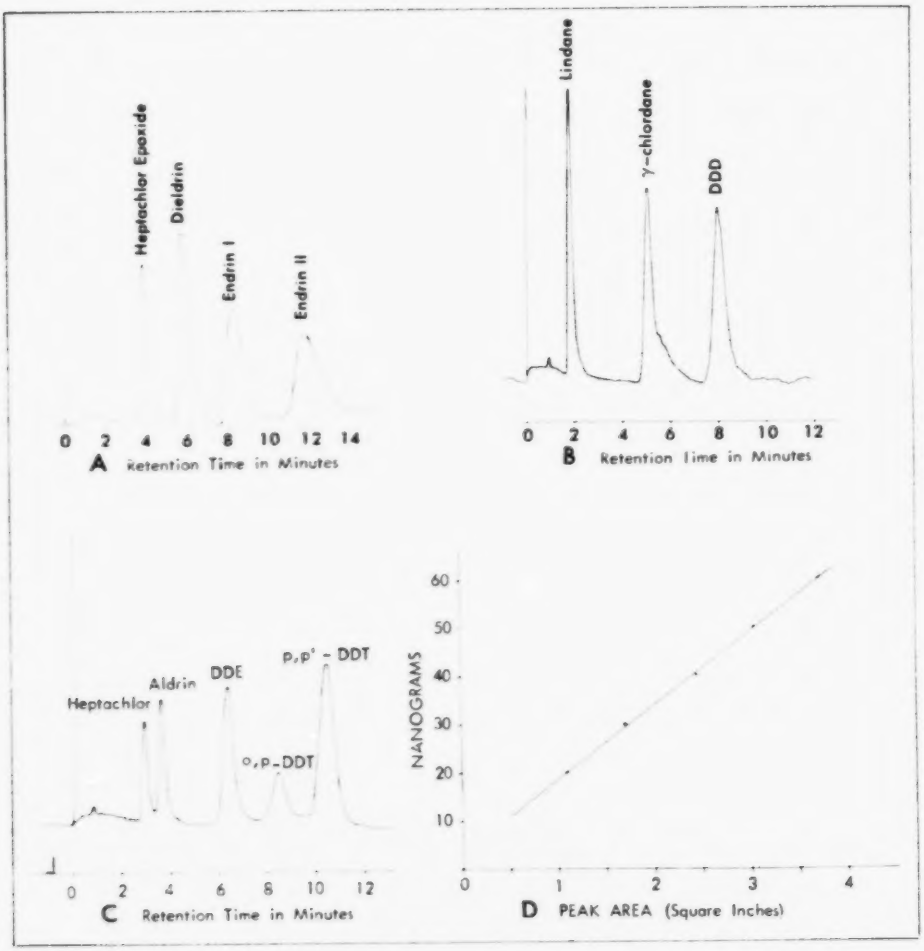
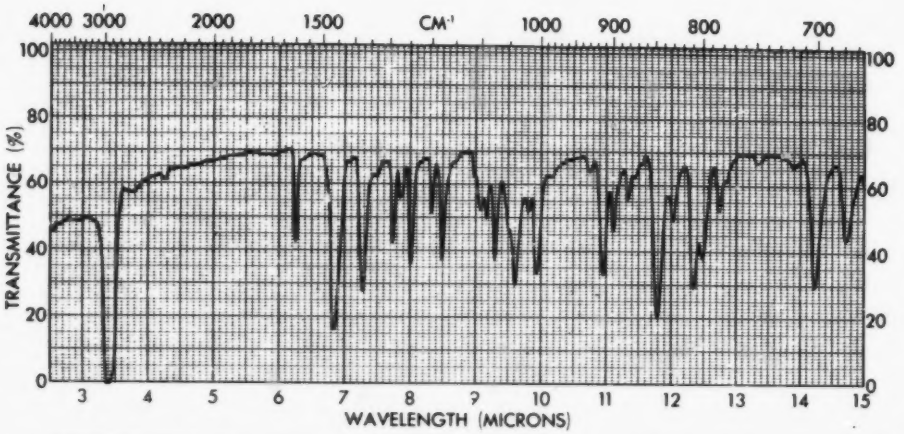


Figure 29

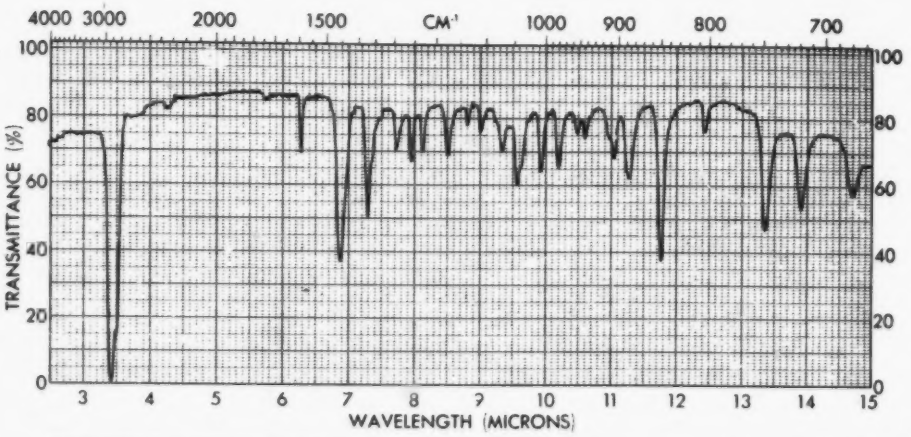
MCT Gas Chromatograms of Standard Pesticides in (A) TLC Section II (heptachlor epoxide—25 ng, dieldrin—50 ng, endrin—100 ng), (B) TLC Section III (lindane—20 ng, γ-chlordane—40 ng, DDD—60 ng), (C) TLC Section IV (heptachlor—20 ng, aldrin—20 ng, DDE—40 ng, DDT—100 ng). (D) Sample Calibration Curve for Endrin (MCTGC). See page 30, footnote 31 for operating conditions.



SPECTRUM NO.	ORIGIN NUTRITIONAL BIO-	LEGEND	REMARKS
SAMPLE	CHEMICAL, CLEVELAND, OHIO	1.	
DIELDRIN STANDARD	PURITY 85%	2.	
	PHASE MINERAL OIL MULL	DATE 7/7/64	
	THICKNESS	OPERATOR L.H.	

SPECTRUM NO. SAMPLE

Figure 30. IR Spectrum of Standard Dieldrin in Mineral Oil Mull

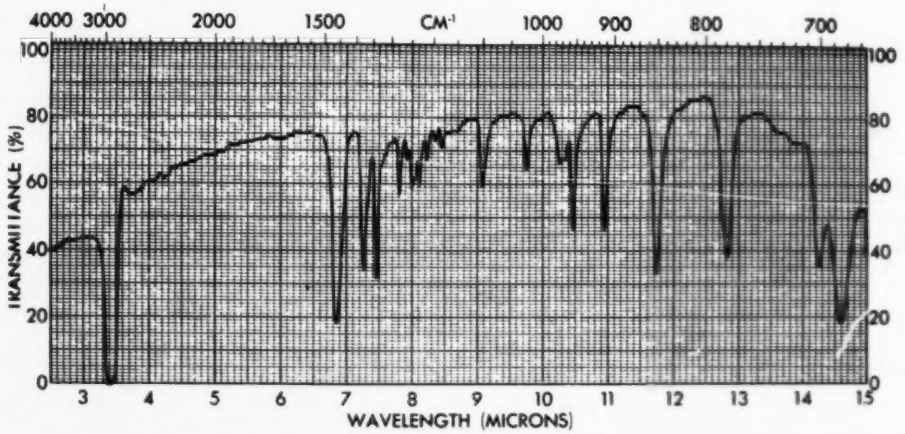


SPECTRUM NO.	ORIGIN NUTRITIONAL BIO-	LEGEND	REMARKS
SAMPLE	CHEMICAL, CLEVELAND, OHIO	1.	
ENDRIN STANDARD	PURITY 96%	2.	
	PHASE MINERAL OIL MULL	DATE 7/7/64	
	THICKNESS	OPERATOR L. H.	

SPECTRUM NO. SAMPLE

Figure 31. IR Spectrum of Standard Endrin in Mineral Oil Mull

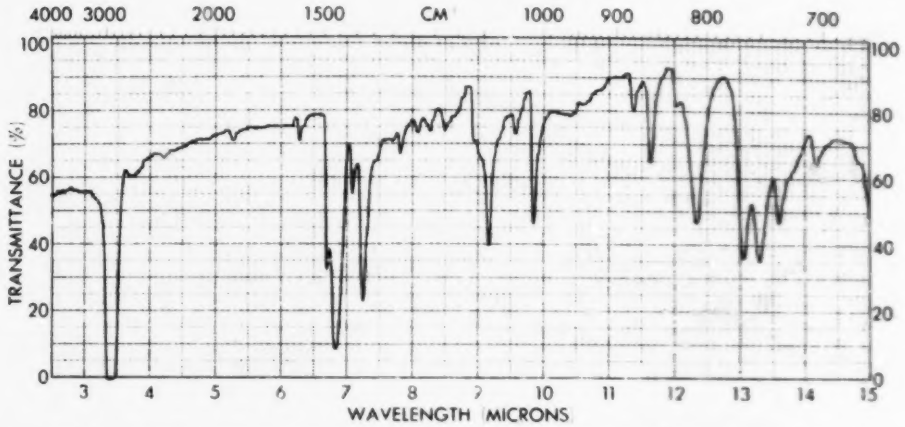
Reproduced from best available copy.



SPECTRUM NO.	ORIGIN NUTRITIONAL BIO-	LEGEND	REMARKS
SAMPLE	CHEMICAL, CLEVELAND, OHIO	1.	
LINDANE STANDARD	PURITY REFERENCE STANDARD	2.	
	PHASE MINERAL OIL MULL	DATE 7/7/64	
	THICKNESS	OPERATOR L. H.	

SPECTRUM NO. SAMPLE

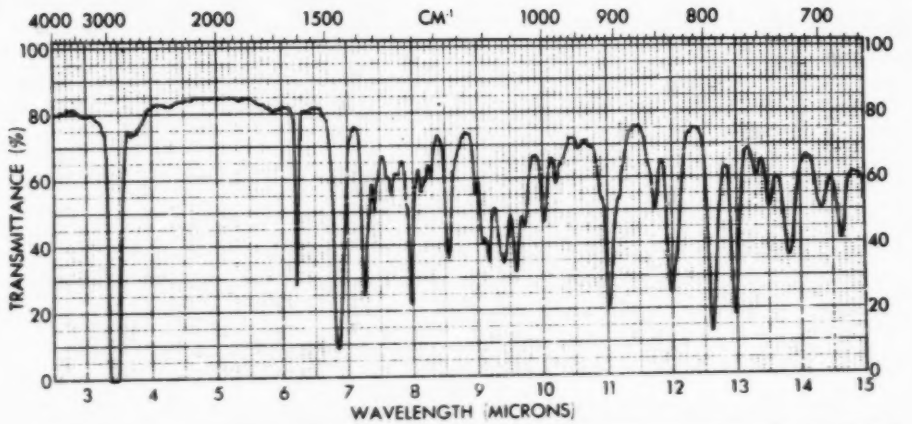
Figure 32. IR Spectrum of Standard Lindane in Mineral Oil Mull



SPECTRUM NO.	ORIGIN NUTRITIONAL BIO-	LEGEND	REMARKS
SAMPLE	CHEMICAL, CLEVELAND, OHIO	1.	
DDD STANDARD	PURITY REFERENCE STANDARD	2.	
	PHASE MINERAL OIL MULL	DATE 7/7/64	
	THICKNESS	OPERATOR L.H.	

SPECTRUM NO. SAMPLE

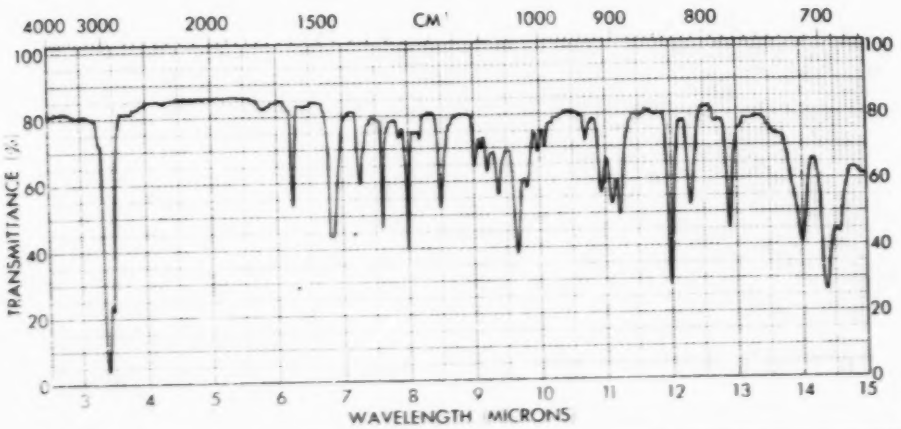
Figure 33. IR Spectrum of Standard DDD in Mineral Oil Mull



SPECTRUM NO.	ORIGIN NUTRITIONAL BIO-	LEGEND	REMARKS
SAMPLE	CHEMICAL, CLEVELAND, OHIO	1.	
HEPTACHLOR STANDARD	PURITY 72%	2.	
	PHASE MINERAL OIL MULL	DATE 7/7/64	
	THICKNESS	OPERATOR L.H.	

SPECTRUM NO. SAMPLE

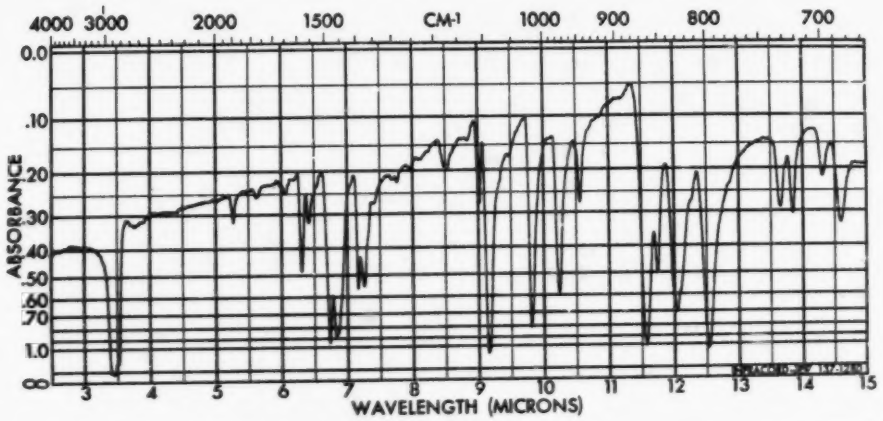
Figure 34. IR Spectrum of Standard Heptachlor in Mineral Oil Mull



SPECTRUM NO.	ORIGIN NUTRITIONAL BIO-	LEGEND	REMARKS
SAMPLE	CHEMICAL, CLEVELAND, OHIO	1.	
ALDRIN STANDARD	PURITY 94%	2.	
	PHASE MINERAL OIL MULL	DATE 7/7/64	
	THICKNESS	OPERATOR L. H.	

SPECTRUM NO. SAMPLE

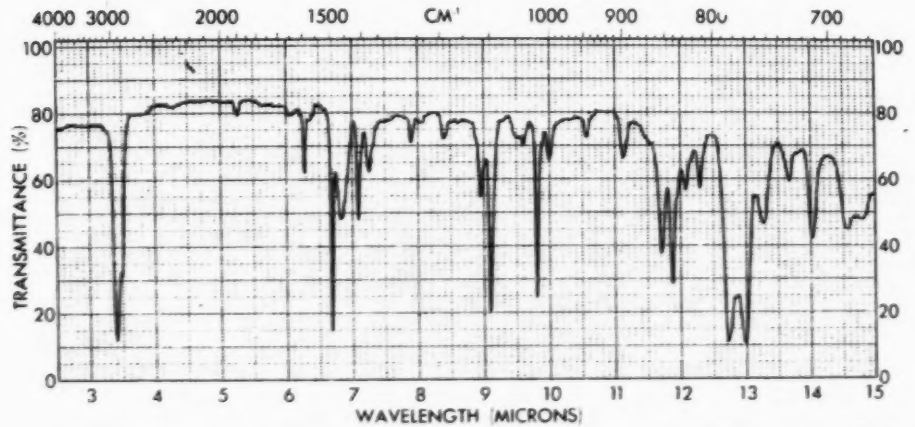
Figure 35. IR Spectrum of Standard Aldrin in Mineral Oil Mull



SPECTRUM NO.	ORIGIN GEIGY AGRICULTURAL CHEMICALS DIVISION	LEGEND	REMARKS
SAMPLE DDE	NEW YORK	1.	
STANDARD	PURITY 99.99%	2.	
	PHASE MINERAL OIL MULL	DATE 7/7/64	
	THICKNESS	OPERATOR L.H.	

SPECTRUM NO. SAMPLE

Figure 36. IR Spectrum of Standard DDE in Mineral Oil Mull



SPECTRUM NO.	ORIGIN NUTRITIONAL BIOCHEMICAL, CLEVELAND, OHIO	LEGEND	REMARKS
SAMPLE		1.	
DDT STANDARD	PURITY 77.2% p,p'-isomer	2.	
	PHASE MINERAL OIL MULL	DATE 7/7/64	
	THICKNESS	OPERATOR L.H.	

SPECTRUM NO. SAMPLE

Figure 37. IR Spectrum of Standard DDT in Mineral Oil Mull

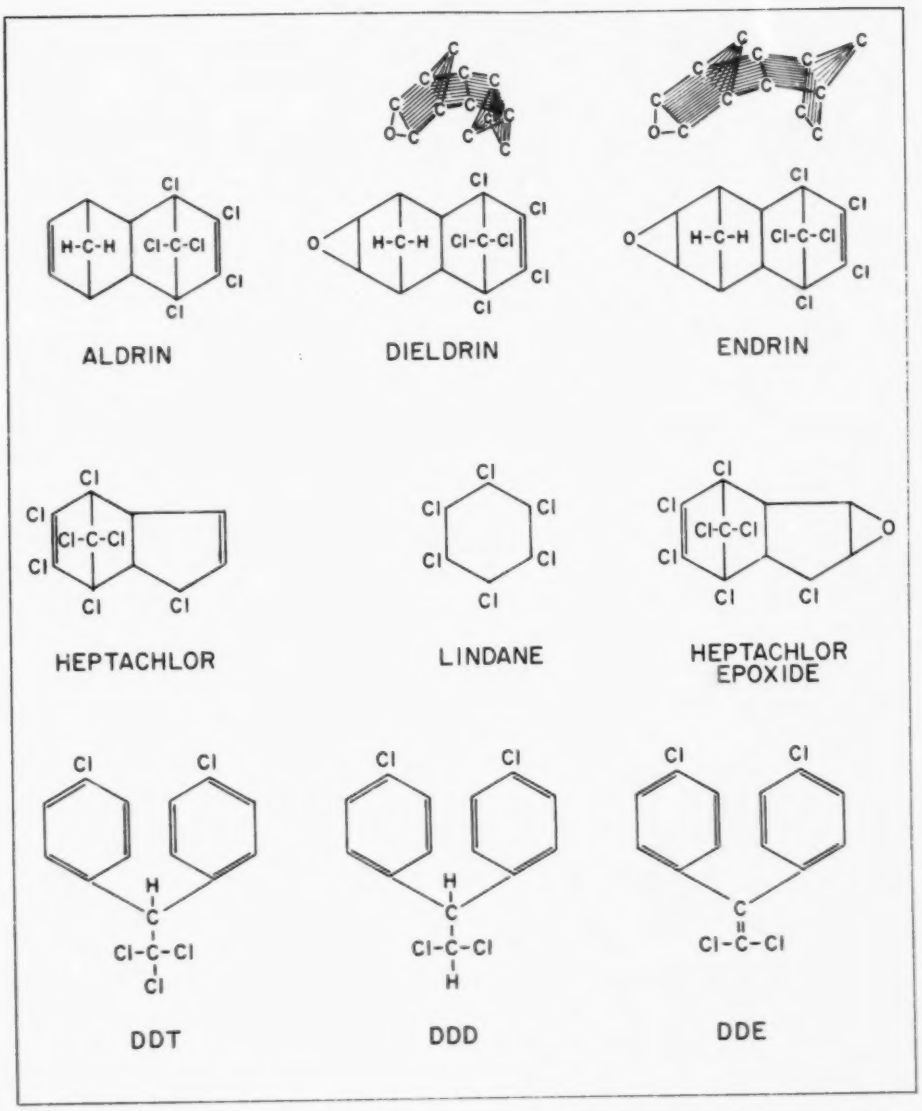


Figure 38. Structural Formulae of Nine Chlorinated Hydrocarbon Pesticides

TABLE 1

R_f VALUES OF PESTICIDES DEVELOPED WITH CCl_4 ON SILICA GEL-G THIN LAYER PLATE

Pesticide	R_f Value	Zone
Methyl Parathion	0.05	II
Parathion	0.07	
Dieldrin	0.17	
Endrin	0.20	
Heptachlor Epoxide	0.29	
Lindane	0.37	III
DDD	0.54	
γ-Chlordane	0.55	
Heptachlor[a]	0.67	IV
DDT	0.68	
DDE	0.72	
Aldrin	0.73	

[a] Technical grade heptachlor contains approximately 30% γ-chlordane.

TABLE 2

GAS CHROMATOGRAPHIC RETENTION DATA

Temperature	Electron Capture				Microcoulometric	
	180°C		195°C		190°C	
Pesticide	Retention Time In Minutes	Relative [a] Retention Time	Retention Time In Minutes	Relative [a] Retention Time	Retention Time In Minutes	Relative [a] Retention Time
Lindane	1.81	0.43	0.94	0.46	1.90	0.50
Methyl parathion	3.24	0.77	—	—	—	—
Heptachlor	3.24	0.77	1.58	0.78	3.07	0.81
Aldrin	4.19	1.00	2.03	1.00	3.80	1.00
Parathion	4.66	1.11	—	—	—	—
Heptachlor epoxide	5.58	1.33	2.61	1.29	4.72	1.24
γ-Chlordane	6.38	1.52	3.00	1.48	5.40	1.42
Dieldrin	8.72	2.08	3.87	1.91	6.95	1.83
DDE (p, p')	8.81	2.10	3.94	1.94	6.79	1.79
DDD	11.71	2.79	4.96	2.44	8.87	2.33
DDT (o, p)	12.12	2.89	5.20	2.56	9.09	2.39
DDT (p, p')	15.85	3.78	6.62	3.26	11.39	2.99
Endrin [b]	12.84–19.43	3.06–4.64	5.20–7.40	2.56–3.64	9.63–13.65	2.53–3.59

[a] Ratio of absolute retention time for compound to that of aldrin.

[b] The multiple peaks for endrin have been associated with the thermal isomerization of endrin on gas chromatographic columns (*63*).

TABLE 3

SOME COLUMN PACKINGS USED FOR GAS CHROMATOGRAPHIC ANALYSIS OF CHLORINATED HYDROCARBON PESTICIDES

Solid Support	*Liquid Phase*	*Reference*
Chromosorb W (60-80 mesh)	5% Dow 11 Silicone	(*14*)
Chromosorb W (60-80 mesh)	10% SE-30 Silicone Gum	(*45*)
Chromosorb W (60-80 mesh)	5% FS-1265 (QF-1) Fluoro Silicone	(*21*)
Chromosorb W (60-80 mesh) acid washed	4% SE-30 Silicone Gum + 6% FS-1265 (QF-1) Fluoro Silicone	(*64*)
Chromosorb W (60-80 mesh) silanized (HMDS)	2.5% Dow 11 Silicone	(*65*)
Chromosorb W (100-120 mesh) silanized (HMDS)	5% Apiezon-L	(*20*)
Chromosorb W (45-60 mesh) silanized (HMDS)	5% SE-30 Silicone Gum	(*20*)
Chromosorb P (60-80 mesh) acid washed	5% DC-200 Silicone	(*46*)
Chromosorb P (30-60 mesh) acid washed	15 to 30% High Vacuum Silicone Grease	(*52*) (*66*)
Chromosorb P (30-60 mesh) acid washed	20% SE-30 Silicone Gum	(*67*)
Chromosorb P (30-60 mesh) acid washed	2.5% Epon Resin 1001	(*68*)
Chromosorb P (60-80 mesh) acid washed	5% FS-1265 (QF-1) Fluoro Silicone + 3% DC-200 Silicone	(*69*)
Fluoropak-80 preceded by CaC_2	12% Dow 11 Silicone + 6% Epon 1001	(*47*)
Anakrom ABS (90-100 mesh)	10% DC-200 Silicone	(*70*)
Gas Chrom Q (100-120 mesh)	10% DC-200 Silicone	(*71*)

APPENDIX THREE

EQUIPMENT, SOLVENTS AND REAGENTS

I. EQUIPMENT, SOLVENTS AND REAGENTS USED TO COLLECT AND PROCESS CCE SAMPLES (PER SAMPLE)

A. EQUIPMENT

Collection equipment (see Appendix One.)
1 Drying oven.
1 1-gallon paint can with crimp lid.
1 Extraction assembly, includes:
- a. 2 3-liter round bottom flasks
- b. 1 large Soxhlet extractor
- c. 1 Friedericks condenser
- d. 1 3-liter heating mantel (Glas-Col Series M)
- e. 1 Variable transformer (Powerstat, Type 116)

1 Manifold for filtering and warming air for drying carbon in Soxhlet.
1 10-ml beaker.
1 50-ml beaker.
1 100-ml beaker.
2 300-ml Erlenmeyer flasks.
12 125-ml Erlenmeyer flasks.
1 125-ml vacuum flask.
1 60° sintered-glass funnel.
11 5-dram glass vials.
1 chromatographic column (19 mm I.D. with medium porosity fritted disc).
1 100-ml graduated cylinder.
1 each 10-μl, 50-μl and 100-μl syringes for gas chromatographic injections.

B. SOLVENTS (all redistilled A.R. grade)

3 liters chloroform
3 liters ethanol (95%)
100 ml of methanol
100 ml of isooctane
100 ml of benzene
300 ml of ethyl ether

C. REAGENTS

50 ml HCl (conc.)
50 ml HCl (5%)
50 ml $NaHCO_3$ (5%)
50 ml NaOH (5%)
NaOH (25%) or pellets
20 grams activated silica gel (Davison Code 950–08–08–226, 60–200 mesh)

D. PESTICIDE STANDARDS

II. EQUIPMENT, SOLVENTS AND REAGENTS USED TO COLLECT AND PROCESS WATER GRAB SAMPLES (PER SAMPLE)

A. EQUIPMENT

1 1-quart glass bottle with Teflon liner in cap.
1 Constant temperature water bath (40°C).
Compressed air source (clean-dry).

1. Semi-Automatic Extraction

1 Semi-automatic extractor includes:
1 Teflon impeller containing bar magnet (Teflon is used because it does not contaminate the solvents and is highly resistant to corrosive cleaning agents).
1 Bottle lid with the center removed and a double Teflon liner inserted.
1 Magnetic stirrer.
1 Teflon reclamation plug with glass recovery tube.
1 Pressure device to secure reclamation plug.
1 Source of compressed air.
1 50-ml graduated cylinder.
1 100-ml beaker.
1 1000-ml graduated cylinder.
1 15-ml graduated centrifuge tube.

2. Separatory Funnel Extraction

1 2-liter separatory funnel with Teflon stopcock.
1 1500-ml beaker.
2 300-ml Erlenmeyer flasks.
1 Chromatographic column (19 mm I.D.).
1 each, 10-μl, 50-μl, and 100-μl syringes for gas chromatographic injections.
1 Kuderna-Danish Evaporator with ampoule graduated in 0.01 ml divisions.

B. SOLVENTS (CHROMATOGRAPHIC GRADE)

1. Semi-automatic Extraction
50 ml hexane-benzene (1:1)

2. Separatory Funnel Extraction
300 ml of hexane.

C. REAGENTS

Anhydrous sodium sulfate.

III. EQUIPMENT, SOLVENTS AND REAGENTS USED TO PROCESS BOTTOM SAMPLES

Each extraction apparatus includes:

1 300 ml round bottom flask Ŧ 24/40.

1 Soxhlet extractor, 40 mm I.D. x 205 mm length, bottom joint Ŧ 24/40, top joint Ŧ 45/50.

1 Allihn condenser joint Ŧ 45/50

Pre-extracted glass wool.

1 Heating mantle, 300 ml.

200 ml of hexane-acetone (9:1).

1 Large porcelain mortar and pestle.

IV. EQUIPMENT, SOLVENTS AND REAGENTS FOR THIN LAYER CHROMATOGRAPHY

Plates, 200 mm x 200 mm (8" x 8") glass

Chamber, glass developing, with lid, 8½" x 4" x 8½"

Spreader, variable thickness

Thickness gauge

Plate holder, plastic

Plate carrier

Spotting template

Chromatography sprayer

Desiccator to accommodate 200-mm plates

UV light box

Micropipets, 1μl—10 μl, 100 μl

Eye droppers

Spatulas

Graduated centrifuge test tubes, capacity 15 ml

Glass wool, pre-extracted with chloroform

Pesticide standards

All pesticides 0.1% w/v (1 mg/ml) in hexane

Chromogenic agents:

Rhodamine B base, spirit soluble 0.10 mg/ml in ethanol

Silver nitrate solution

1.7 g $AgNO_3$ in 5 ml distilled water is added to 10 ml 2-phenoxyethanol (MC & B, technical), and diluted to 200 ml with acetone.

5% bromine in carbon tetrachloride

Fluorescein solution

MC & B fluorescein, water soluble, U.S.P., 25 mg. in 100 ml dimethylformamide; 10 ml of this concentrated solution are diluted to 50 ml with ethanol 95% for spraying.

Adsorbents:

Silica Gel G

Aluminum Oxide G

Solvents:

All solvents are redistilled before use

Hexane

Carbon Tetrachloride

Ethyl ether-petroleum ether (1:1)

Acetone

Dye Mixture:

Sudan Yellow, Sudan IV, Azobenzene, 0.1% in benzene

APPENDIX FOUR

General Composition of Carbon Chloroform and Carbon Alcohol Extracts

I. CHLOROFORM EXTRACTS

The organic residue recovered from the carbon adsorption column by chloroform is very complex. It is desirable to separate the crude extract into certain broad chemical classes, and this can be done on the basis of solubility differences. The various classes or groups and their general significance are discussed briefly below.

A. ETHER SOLUBLES

This group is usually a brown, humus-like powder, apparently composed to a large extent of carboxylic acids, ketones, and alcohols of complicated structure. Origin of the group, which is an indicator of "old" pollution, is believed to be partially oxidized sewage and industrial wastes. For example, the Ohio River at Cincinnati has been exposed to much industrial and sewage pollution, and hence large amounts of ether-insoluble materials are found. Streams with little or no pollution history have little or no ether insolubles. Chloroform extracts contain from 0 to 30 percent of ether-insoluble material.

B. WATER SOLUBLES

These substances are largely acidic and undistillable at moderate temperatures, but their solubility in ether indicates that the molecules are smaller and probably simpler than the ether-solubles. On the other hand, their water solubility practically requires the presence of several functional groups, such as hydroxy-acid, keto-acid, and keto-alcohol. Such compounds probably originate from partial oxidation of hydrocarbons or they may be natural substances. They have very little odor. These materials usually make up 10 to 20 percent of the total extract.

C. WEAK ACIDS

This group is characterized by being removed from ether solution with sodium hydroxide but not with sodium bicarbonate. Phenols are the best known weak acids, and if present in the water, appear in this group. Other weakly acidic compounds include certain enols, imides, sulfonamides, and some sulfur compounds. This group of materials also occurs in nature. The weak acids are odorous, and commonly constitute 5 to 20 percent of the chloroform extract.

D. STRONG ACIDS

These acids are usually carboxylic acids such as acetic, benzoic, salicylic or butyric. Although classified as strong in reference to carbonic acid, they are actually weak when compared with a mineral acid, such as sulfuric. Many of the compounds are used industrially, but may also be produced by natural processes, such as fermentation. Some of the materials are highly odorous. This fraction makes up from 5 to 20 percent of the total. The significance of the strong acids can be interpreted only in the light of stream pollution conditions.

E. BASES

These compounds are organic amines. Such materials as aniline and pyridine are amines of commerce. Lower amines may occur as a result of decomposition. Although odorous, the lower concentrations found are not likely to cause objectionable conditions. However, in the case of specific amine-containing wastes the compounds can be of considerable significance. Generally, only 1 or 2 percent of the total extract is made up of the bases.

F. NEUTRALS

This group frequently constitutes the major portion of the chloroform extract. Neither basic nor acidic, the materials are less reactive and tend to persist in streams longer than many other types. Hydrocarbons, aldehydes, ketones, esters, and ethers are examples of neutral materials. The group lends itself to further fractionation by chromatographic separation into aliphatic, aromatic, and oxygenated subgroups:

1. Aliphatics:

This portion represents petroleum-type hydrocarbons in a considerable state of purity, and is usually made up of mineral oil type of material. The percentage of aliphatics present yields important information about the possible source of pollution, since petroleum is the most likely source.

2. Aromatics:

These are principally the coal tar hydrocarbons such as benzene, toluene, and a host of others, and their presence in any significant amount is a reliable indication of industrial pollution. Further, the materials can frequently be identified by infrared spectrophotometry. Some aromatic compounds which have been found in our rivers—and in our drinking water—include DDT, aldrin, dieldrin, endrin, phenyl ether, orthonitrochlorobenzene, pyridine, phenol, and others. Some of these materials are highly odorous; others may also be toxic. Their appearance in any quantity as pollutants should receive careful evaluation.

3. Oxygenated Compounds (Oxys):

These are the neutral compounds containing oxygen in aldehyde, ketone, or esters groups. They may have originated by direct discharge or may represent oxidation products from both natural and industrial materials. They help to indicate the "age" of the pollution, since pollution exposed to oxidation forces for a long time would be expected to contain large amounts of oxys. The oxy materials are odorous.

G. LOSSES

Manipulative losses inherent in this type of separation may amount to 10 to 15 percent. Losses greater than this may indicate that volatile components were lost from the sample. Such volatiles may have significance as pollutants.

II. ALCOHOL EXTRACTS

The alcohol extractables generally consist of materials more polar than the chloroform extractables. They often contain synthetic detergents, carboxylic acids and humic materials which may originate naturally or from oxidized products of domestic and industrial wastes. These classes of substances are not quantitatively recovered by the alcohol extraction. For example, this extraction recovers only 20 to 30 percent of the synthetic detergents present. On waters of mixed industrial and domestic pollution, the chloroform and alcohol extractables may be about equal. On some streams where the industrial pollution is rather low and much natural pollution or sewage is present, the alcohol extractables may exceed the chloroform extractables by a factor of 4 to 6.

The alcohol extract is usually only partially soluble in water and most ordinary solvents. Very little further chemical separation of this material is currently practical. However, tests have revealed that synthetic detergents may make up 1 to 12 percent of the alcohol extract.

APPENDIX FIVE

GLOSSARY

mg	milligram (10^{-3} gram)
μg	microgram (10^{-6} gram)
ng	nanogram (10^{-9} gram)
pg	picogram (10^{-12} gram)
ml	milliliter (10^{-3} liter)
μl	microliter (10^{-6} liter)
CAM	carbon adsorption method
CAC	carbon adsorption column
CCE	carbon chloroform extract
CAE	carbon alcohol extract
EI	ether insolubles
WS	water solubles
B	bases
SA	strong acids
WA	weak acids
N	neutrals
AL	aliphatics
AR	aromatics
OXY	oxygenated substances
IR	infrared
TLC	thin layer chromatography
GC	gas chromatography
ECGC	electron capture gas chromatography
MCTGC	microcoulometric titration gas chromatography
Rf	$\frac{\text{distance travelled by a given substance}}{\text{distance travelled by solvent front}}$

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